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Hepatol Int (2008) 2:S37–S211
DOI 10.1007/s12072-008-9079-9

Welcome Message

Dear Colleague,

It is our pleasure to invite all of you to the 4th Hong Kong – Shanghai International Liver Congress (ILC) 2008, which to be held on 12th – 15th June 2008 at the Hong Kong Convention and Exhibition Center, Hong Kong, China.

This year, the theme of the meeting is "Molecular Targeting in Liver Disease – from Bench to Bedside" and we make every effort to captures the latest developments in hepatology. The three-and-a-half day programme will be commenced with a half-day postgraduate course which jointly organized by the Clinical Trial Centre of the University of Hong Kong, Liver Cancer Institute of Zhong Shan Hospital and the Mayo Clinic of Rochester, USA. Distinguished scholars from all over the world will present their latest findings and unique experience in the following days of programme, which will provide with a wide spectrum of topics and issues in liver disease. Moreover, you will find various industrial sponsored symposia and workshops. While the scientific programme serves as a platform for academic sharing between healthcare professionals and scholars, the exhibition will give you a showcase of the latest development and technology from the industry. We are sure that the meeting will satisfy your appetite for learning.

As Hong Kong is always described as "the window of China", we believe this meeting will enhance the interaction between Chinese and international hepatologists. We sincerely welcome you all to this wonderful meeting. See you there!

Yours truly,

Zhao-you Tang
Chairman

Shiu-Kum Lam
Chairman

George K.K. Lau
Secretary General

Hui-Chuan Sun
Secretary General

Acknowledgements

Hong Kong – Shanghai International Liver Congress 2008 would like to thank the following individuals who served as reviewers for abstracts submitted to the ILC 2008:

Nezam H. Afdhal Boston
Steven R. Alberts Rochester
Maria Buti-Ferret Barcelona
Tai-Nin Chau (周泰年) Hong Kong
Min-Shan Chen (陈敏山) Hong Kong
Pei-Jer Chen (陈培哲) Taipei
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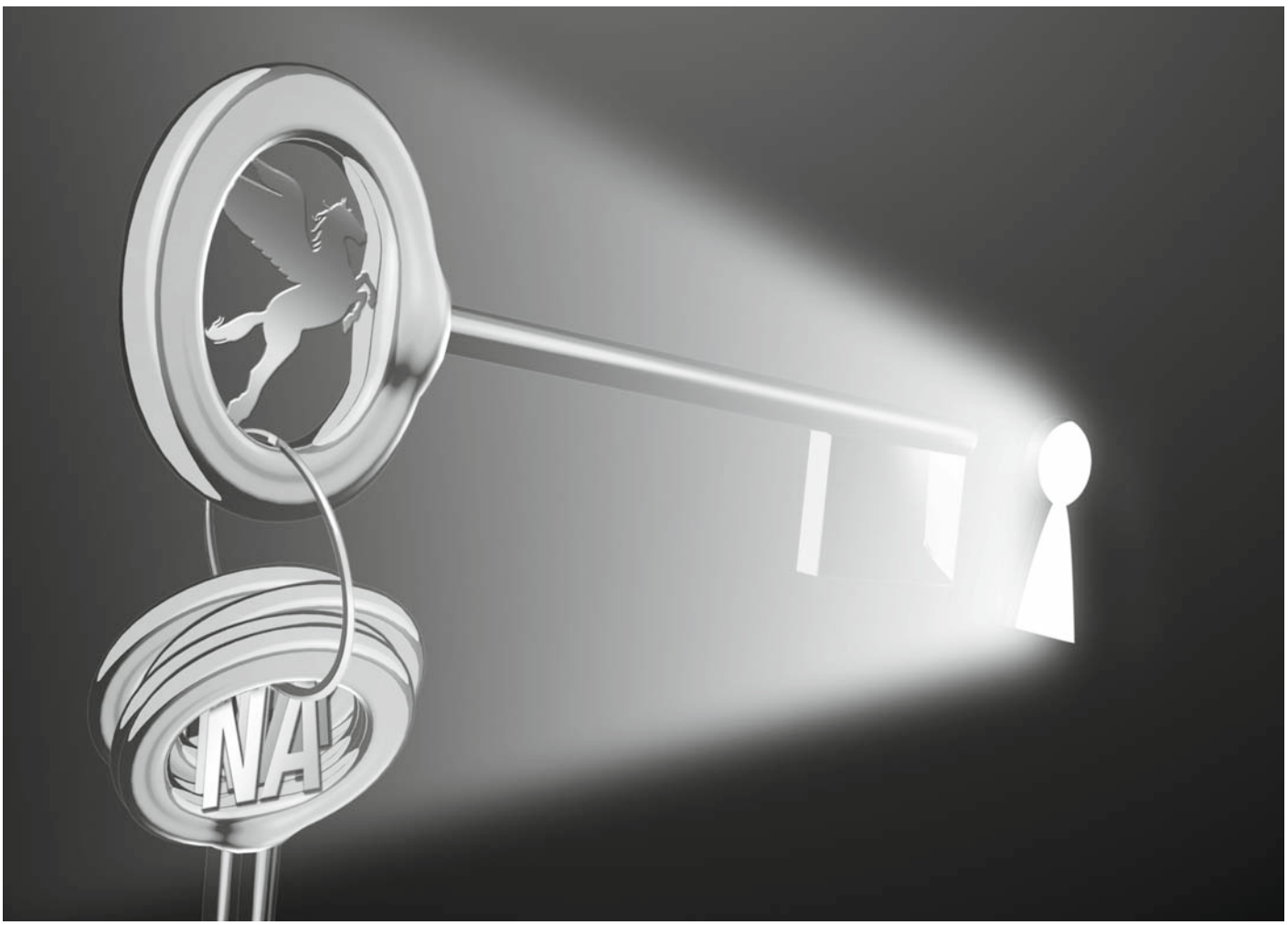
Management of Chronic Hepatitis B – Making the Right Decisions



Symposium
13:00–14:30, Sunday, June 15, 2008
Convention Hall A, Hong Kong Convention and Exhibition Center, Hong Kong

Hong Kong - Shanghai International Liver Congress 2008 (ILC2008)
12 - 15 June 2008, Hong Kong Convention and Exhibition Center, Hong Kong, China

Plenary Session

PL-01

Randomized phase III trial of sorafenib versus placebo in Asian patients with advanced hepatocellular carcinoma

Ann-Lii Cheng[1], Yoon-Koo Kang[2], Zhendong Chen[3], Chao-Jung Tsao[4], Shukui Qin[5], Jun Suk Kim[6], Karin Burock[7], Jessie Zou[8], Dimitris Voliotis[9], Zhongzhen Guan[10]

[1] National Taiwan University Hospital, Taipei, Taiwan 10016, [2] ASAN Medical Center, Songpa-gu, Seoul, Republic of Korea, [3] First Affiliated Hospital of Anhui Medical University, Hefei, China, [4] Chi Mei Medical Center, Liou Ying Campus, Tainan, Taiwan, [5] Nanjing 81 Hospital, Nanjing, China, [6] Korea University Guro Hospital, Seoul, Republic of Korea, [7] Bayer Vital GmbH, Leverkusen, Germany, [8] Bayer Schering Pharma, Shanghai, China, [9] Bayer Schering Pharma, Wuppertal, Germany, [10] Sun Yat-sen University Cancer Center, Guangzhou, China

Background: We report efficacy and safety results of a phase III trial of sorafenib in HCC patients from the Asia–Pacific region.

Methods: Treatment–naïve patients with advanced HCC (ECOG PS 0–2, CP–A) were randomized 2:1 (sorafenib 400mg bid [n=150]: placebo [n=76]). Endpoints were overall survival (OS), time-to-progression (TTP), time-to-symptomatic-progression (TTSP), disease control rate (DCR)*, and safety.

Results: Efficacy data are tabulated below. DCR was 35% (95% CI 28–44) and 16% (95% CI 8–26) for sorafenib vs placebo. Most frequently reported grade ≥3 drug-related AEs were (sorafenib/placebo): hand–foot skin reaction (10.1/0%), diarrhea (6.0/0%), hyperbilirubinemia (3.4/2.7%) and fatigue (3.4/1.3%). Drug-related serious AEs were observed in 13 (9%) and 1 (1%) patients (sorafenib vs placebo).

Conclusions: Sorafenib significantly prolonged OS and TTP in HCC patients from the Asia-Pacific region vs placebo. The sorafenib safety profile in these patients was comparable with the previously reported phase III SHARP trial.

Endpoint	Events (n [%], Sorafenib/Placebo)	HR (95% CI)	Median (months, Sorafenib/Placebo)	*P*-value (log-rank)
OS	102 (68)/62 (82)	0.68 (0.49–0.93)	6.2/4.1	0.0155
TTP	108 (72)/58 (76)	0.58 (0.42–0.80)	2.8/1.4	0.0007
TTSP	126 (84)/65 (86)	0.89 (0.66–1.20)	3.5/3.4	0.4458
PFS	134 (89)/73 (96)	0.62 (0.46–0.83)	2.8/1.4	0.0009
* DCR: Complete or partial response, or stable disease according to RECIST, maintained for ≥28 days from first demonstration				

PL-02

Differential regulation of hepatitis B virus core promoter/enhancer II by TAp73 and its oncogenic counterpart DNp73: implications for hepatocarcinogenesis

Sven Buhlmann[1], Tomas Racek[1], Katja John[1], Stephan Schaefer[2], Brigitte M. Putzer[1]

[1] Vectorology & Experimental Gene Therapy, Biomedical Research Center, University of Rostock, Rostock, Germany, [2] Institute of Virology, University of Rostock, Rostock, Germany

Hepatitis B virus (HBV) is a causative agent of chronic hepatitis and hepatocellular carcinoma (HCC). In contrast to p53, the homologue p73 gene is rarely mutated and overexpression of different p73 isoforms in HCC has been observed. Previous studies reported repression of HBV replication by p53 and full-length p73. However, the exact mechanism of this repressive function is unknown. In the present study, we investigated the regulation of the HBV enhancer II/core promoter (Enh II) by transactivation competent TAp73beta and its oncogenic isoform lacking the N-terminal transactivation domain (DNp73). Overexpression of TAp73beta lead to a significant repression of Enh II activity in p53-negative Hep3B hepatoma cells. In contrast, enhanced expression of transactivation deficient p73 resulted in Enh II activation. Moreover, p73-mediated repression of HBV transcription was substantially abolished by DNp73. We demonstrated that p73 directly binds to the Sp1 transcription factor, known to be a key stimulator of HBV gene expression, thereby removing Sp1 from Enh II. This result suggests that formation of a p73-Sp1 complex is one of the underlying mechanisms for p73-triggered inhibition of the HBV enhancer II/core promoter. Additionally, chromatin immunoprecipitation (ChIP) revealed direct promoter binding of p73, indicating a dual repressive mechanism by which p73 controls expression of HBV proteins. Our data provide new insight in the regulation of hepatitis B virus replication by the tumor suppressor p73 and its oncogenic counterpart DNp73.

This work was supported by the FORUN program of the Medical Faculty of Rostock University.

PL-03

Functional characterization of truncated hepatitis HBX mutant protein

Waye Mary Miu Yee[1,2], Li Sheila SK[3]

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Chronic hepatitis B virus (HBV) infection has been one of the major causes of hepatocellular carcinoma. This virus encodes a protein a multifunctional protein, HBx which acts as an atypical transactivator in cells, induces apoptosis and regulates cell growth. However, the reported mechanisms of HBx in modulating apoptosis are mostly controversial and ill-demonstrated. Like many other proteins produced by tumor viruses, HBx localizes to mitochondria which is suspected to be the site of action for bringing out the apoptotic effect. While working as an integrated genome, HBx found in chronic carriers is truncated at the termini. To investigate the effect of truncation in altering HBx function, we generated a series deletion mutants, with deletions at different sites of the termini. Overexpression of GFP fusion protein induces a perinuclear mitochondrial localization of one type of mutant in a pattern similar to full-length HBx. Deletion at another site in another mutant abrogates the mitochondrial localization of this viral protein. This implies that truncations in HBx protein in chronic HBV carriers can abrogate its mitochondrial localization and thus alter its roles in affecting cellular functions. Other biochemical characterization of these mutants will be also be discussed. Finding out the role of HBX in liver cell transformation will give us more insight into the mechanism of how hepatitis B virus infection could lead to an increased risk of liver cancer.

PL-04

Prevention strategies using Anti-HBc positive liver donation

Robert Perrillo[1]

[1] Baylor University Medical Center

Background: Optimal prophylaxis for anti-HBc (+) liver donation remains unknown.

Methods: 76 international transplant centers were queried as to the following: (1.) Is LAM used to prevent reactivation? (2.) If not, what nucleoside analog (NA) is used? (3) How long is this given? (4) Is HBIg used? (5) If so, how long and by what route?

Results: 50 US, 19 European, 5 Canadian, and 2 Asian/Australian centers responded. All use NA therapy. 60 sites (79%) always use LAM, an additional 11 (14%) use LAM sometimes and 5 (7%) never use it. 28 sites (37%) always use HBIg, 16 (21%) use it sometimes, and 32 (42%) never use HBIg. The vast majority of sites use long term NA but wide disparity is observed relative to HBIg . NA is given indefinitely in 78% and for 6 -12 months in 17%. Of the 44 sites using HBIg, this is given anhepatically only in 18%; anhepatic and wk 1 in 7%; IV or IM for 6-12 mos in 43%; and indefinitely in 7%.
Conclusions: (1.) While NA use is universal, ≈ 20% do not use it long term. (2.) Many use HBIg with considerable variation in treatment duration (3.) A multinational study of NA with / without HBIg, is needed and would be particularly relevant in countries with high HBV prevalence.

PL-05

Serial monitoring of viral load and serum alanine aminotranferase level and the risk of hepatocellular carcinoma: R.E.V.E.A.L.-HBV Study Update

Chien-Jen Chen[1,2], Hwai-I Yang[1,2], Jun Su[3], Chin-Lan Jen[1,2], San-Lin You[1,2], Chuen-Fei Chen[1,2], Uchenna H Iloeje[3] for the R.E.V.E.A.L.-HBV Study Group
[1] *Genomics Research Center, Academia Sinica, Taipei, Taiwan,* [2] *Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan,* [3] *Global Epidemiology and Outcomes Research, Research and Development, Bristol-Myers Squibb Co, Wallingford, USA*

Objective The R.E.V.E.A.L.-HBV study showed baseline viral load predicts hepatocellular carcinoma (HCC) risk. Now using complete data, we evaluate the importance of serial HBV DNA and alanine aminotransferase (ALT) monitoring in HCC risk.
Methods Participants (N=3,584) were HBsAg-seropositive, anti-HCV-seronegative and without liver cirrhosis at entry. Baseline samples were used for all patients and follow-up samples for patients with baseline HBV DNA $\geq 10^4$ copies/mL (N=1,564). Data linkage to the National Cancer Registry and Taiwan Death Certificate's profiles were used to confirm new HCC cases against set criteria. Multivariable adjusted hazard ratios (HR_{adj}), including time-independent baseline and time-dependent follow-up serum HBV DNA levels, were derived using time-dependent Cox's proportional hazard models.
Results A crude incidence rate of 305.5 new HCC cases per 100,000 person-years was seen (total 42,878 person-years of follow-up). An adjusted regression model showed a trend towards greater HCC risk with increasing ALT and HBV DNA levels ($P<0.001$). The HR_{adj} (95% CI) were 4.5 (1.3–15.2), 3.6 (1.1–12.3), 4.2 (1.2–14.5) and 7.3 (2.3–23.5) for HBV DNA levels (baseline and follow-up) 300–9,999; 10,000–99,999; 100,000–999,999; and $>10^6$ copies/mL, respectively. The HR_{adj} (95% CI) for serum ALT levels 16–44 and ≥45 U/L were 1.8 (1.2–2.8) and 3.8 (2.3–6.5), respectively.
Conclusions Increasing HBV DNA levels remain a significant predictor of HCC once follow-up HBV DNA and serum ALT are considered. Furthermore, persistence of high HBV load leads to the highest HCC risk. Long-term monitoring of HBV viral load is essential for chronic hepatitis B management.

PL-06

Changes of Protein Expression Profiles after Hepatitis B Virus Transfection and Interferon alpha Treatment in Human Liver Cell Line HepG2

Jiang Hua Wang[1], Sa Lv[1], Feng Liu[1], Ran Fei[1], Lai Wei[1]
[1] *Peking University Hepatology Institute, Peking University People's Hospital*

Background and aims To investigate the global proteomic responses of liver-derived cells to Hepatitis B virus (HBV) infection and antiviral mechanism of interferon alpha (IFNα).
Methods 2-DE and mass spectrometry-based analysis was performed to compare the proteome changes between HBV stably transfected HepG2.2.15 cell line and its parental cell line HepG2, as well as HepG2.2.15 before and after interferon alpha treatment (5000IU/ml for 48 hours).
Results Compared to HepG2, 12 of 18 down-regulated and 25 of 32 up-regulated proteins were identified in HepG2.2.15. After IFNα treatment, 6 of 7 down-regulated and 13 of 14 up-regulated proteins identified. Differentially expressed proteins caused by HBV infection are involved with Cytoskeletal/Extracellular matrix, heat shock and stress, Kinases/signal transduction, Protease/Proteasome components, and Ubiquitination, etc. Interferon induced protein 15 and prohibitin showed a dose-dependent up-regulation during IFNα treatment.
Conclusions HBV infection may cause protein alterations in diverse cellular functional categories. Interferon induced protein 15 and prohibitin may play important roles in antiviral activity of IFNα.

Young Investigator Session

YI-01

Prediction of the disease severity using the serum retinol binding protein, hyaluronic acid, and transferrin in patients with chronic liver disease

Jung Hyun Kwon[1], Sung Won Lee[1], Hyun Young Woo[1], Jin Dong Kim[1], Chan Ran You[1], Jeong Won Jang[1], U Im Chnag[1], Chang Wook Kim[1], Soon Woo Nam[1], Si Hyun Bae[1], Jong Young Choi[1], Seung Kew Yoon[1]

[1] Departmet of internal medicine, College of Medicine, The Catholic University of Korea

Serum hyaluronic acid (HA) was introduced as a useful marker of hepatic fibrosis. Retinol binding protein (RBP), association with the insulin resistance, and transferrin(TF) correlated with the hepatic biosynthetic capacity. The aim of this study was to evaluate the relationship among these serologic markers, several hematologic and biochemical parameters in patients with chronic liver disease (CLD). We analyzed the data of 310 patients (24 control, 106 chronic hepatitis, 146 compensated liver cirrhosis (LC), 34 decompensated LC). HA was measured by enzyme-linked binding protein assay, RBP and TF by immunonephelometric assay. In CLD patients, RBP and TF were significantly reduced compared with control subjects and significantly correlated with the severity of CLD ($p<0.001$) in contrast the HA inversely correlated ($p<0.001$). In the patients with chronic hepatitis, RBP rather than the HA and TF correlated well with the aspartate aminotransferase, albumin, total bilirubin, glutamyl transferase, platelet, prothrombin time, although TF rather than HA and RBP correlated with aspartate aminotransferase, alanine aminotransferase, total bilirubin, prothrombin time in the patients with decompensated LC. Interestingly, there were few parameters correlated with HA, RBP, and TF in control subjects. After antiviral therapy (n=29, median interval 1126 day), RBP and HBV DNA were only significant improving factors among several biochemical parameters ($p<0.05$). In conclusion, our study suggested that HA, RBP and TF were serologic markers of prediction of the disease severity in patients with CLD not the healthy subjects. Also the RBP may be useful as a surrogate marker of the antiviral effect instead of the liver biopsy.

YI-02

HBx Stabilizing Cyclin D1 Protein in vitre

XiangMei Chen[1], FengMin Lu[1], Hui Zhuang[1]

[1] Peking University Health Science Center

Objective: To investigate the effect of hepatitis B virus X protein (HBX) on the stability of cyclin D1.

Methods: To measure the turnover rate of cyclin D1 protein, pCMV-HA-HBx or pEGFP plasmids DNAs were transiently transfected into HepG2 cells. 48 hrs after transfection, cycloheximide was added to block new protein synthesis. Cells were then harvested at 0、30、60、90、120、180min intervals after cycloheximide treated. The rate of cyclin D1 decay was then assayed by direct Western blot using the D1a-specific antibody. In addition, the effect of HBx on the stability of exogenous wild type and T286 phospho-site lacking cyclin D1 were analysized by co-transfection of different dosage of pCMV-HA-HBx, either with pFlex-cyclinD1, pFlex-D1 265-295del, or pFlex-D1T286A into Hek293T cells and following Western blot.

Results: Comparison with control，endogenous cyclin D1 protein in HepG2 cells transfected with pCMV-HA-HBx exhibits an increased half-life. With transfected pCMV-HA-HBx DNA increasing, a greater amount of exogenous cyclin D1 protein was visualized in Hek293T cells co-transfected with pFlex-cyclinD1 as determined by Western blot, but no change of expression level of cyclin D1 265-295del and D1T286A were found in Hek293T cells co-transfected with pFlex-D1 265-295de, or pFlex-D1T286A.

Conclusion: The half-life of cyclin D1 can be extended by HBx. The effect of HBx stabilizing cyclin D1 might be dependent on the phosphorylation of cyclin D1 at Thr-286.

YI-03

Hepatitis B virus induced hfgl2 transcription is dependent on c-Ets-2 and MAPK signal pathway

Meifang Han[1], Dong Xi[1], Yaoyong Zhou[1], Weiming Yan[1], Mingfeng Liu[2], Gary Levy[2], Xiaoping Luo[3], Qin Ning[1]

[1] Department of Infectious Disease, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, [2] University Health Network, University of Toronto, [3] Department of Infectious Disease and Department of Pediatrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology

Fibrinogen–like protein 2 (fgl2)/ fibroleukin plays a pivotal role in the pathogenesis of experimental and human fulminant and chronic viral hepatitis. To define the transcription factor(s) and upstream signal transduction pathways involved in the transcription of human fgl2 (hfgl2) in response to HBV infection, HBc, HBs or HBx protein expression plasmids were cotransfected with an hfgl2 promoter luciferase reporter construct into CHO and HepG2 cells respectively. HBc and HBx proteins, but not HBs protein enhanced hfgl2 transcription in both cell lines. In response to both HBc and HBx, a strong regulatory region from -712 to -568 (relative to the transcriptional starting site) was shown to be responsible for hfgl2 gene transcription. c-Ets-2 translocation to the nucleus in association with hfgl2 expression and shRNA interference of c-Ets-2 expression inhibited hfgl2 gene transcription by 64.8% and 60.0%. c-Ets-2 protein was highly expressed in PBMC and liver tissue from patients with severe chronic hepatitis B (CHB) in contrast to patients with mild CHB. Increased phosphorylation of ERK and JNK was detected in PBMC from patients with severe CHB. Treatment with the ERK inhibitor PD098059 inhibited expression of c-Ets-2 in response to HBc protein, whereas the JNK inhibitor SP600125 inhibited expression of c-Ets-2 in response to HBx protein. *Conclusion:* Here we show that HBc and HBx proteins enhance transcription of hfgl2 dependent on c-Ets-2 and on the ERK and JNK signaling pathways.

YI-04

Insignificant prognostic value of mRNA expression of FOXP3 due to its negative correlation with protein expression and its expression on hepatocellular carcinoma cells

Qiang Gao[1,2], Shuang-Jian Qiu[1,2], Jia Fan [1,2], Jian Zhou[1,2], Xiao-Ying Wang[1,2], Ying-Hong Shi[1,2], Yong-Sheng Xiao[1,2], Min-Jie Ju[1,2], Xiao-Wu Huang[1,2], Jian Sun[1,2]

[1] Liver Cancer Institute, Zhong Shan Hospital and Institutes of Biomedical Sciences, Fudan University, [2] Key Laboratory for Carcinogenesis & Cancer Invasion, the Chinese Ministry of Education

The high mRNA and protein expression of FOXP3, the specific marker for regulatory T cells (Treg), are independent predictors for dismal outcome in human cancers. We and others have previously demonstrated the predictive value of FOXP3-protein+ Treg in HCC patients. However, prognostic significance of FOXP3 mRNA expression remains elusive in HCC. Currently, we determined whether high FOXP3 mRNA expression is prognostically significant in HCC using qRT-PCR. Completely opposite to FOXP3-protein+ Treg, intratumoral high expression of FOXP3 mRNA was an independent indicator for favorable survival and reduced recurrence in HCC. A further investigation revealed a negative correlation between intratumoral Treg number and FOXP3 mRNA expression, strengthening the concepts that great discrepancy exits between

mRNA and protein expression levels, and the activation status rather than merely the mRNA expression level of a transcriptor is of utmost significance. Intriguingly, HCC cell lines showed prominent FOXP3 expression at both mRNA and protein levels. This result was in line with the latest report that FOXP3 expression was clearly detectable in pancreatic carcinoma cell lines and tissues transcriptionally and translationally. Thus, we demonstrated for the first time a negative relationship of FOXP3 mRNA and protein expression in HCC, suggesting whether FOXP3 mRNA might serve as a prognostic marker has to be carefully evaluated. Expression of FOXP3 in HCC may enable tumor cells in vitro to directly demolish T-cell function in addition to indirectly through Treg.

YI-05

STAT3 signaling pathway for the synergistic effect of interleukin-6 on in vivo radiation induced hepatitis B virus reactivation in liver

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Purpose: Hepatitis B virus (HBV) reactivation is one unique pathogenesis in carriers with liver toxicity after radiotherapy for hepatobiliary malignancies. Interleukin-6 (IL-6) mediates *in vitro* radiation (RT)-induced HBV replication. We attempt to delineate *in vivo* synergistic effect and molecular mechanism of IL-6 on RT-induced HBV reactivation.

Methods: HBV transgenic mice were treated with liver RT (4 Gy daily for 5 days) with or without pre-RT/concomitant treatment of IL-6 (400 MU bid for 15 days). Serum HBV DNA was measured by real-time quantitative polymerase chain reaction, while IL-6 concentration was by ELISA analysis. Immunohistochemical staining of mouse liver with HBV core protein and phosphorylated signal transducer and activator of transcription (STAT)3 were qualitatively analyzed. HepG2.2.15 cells (a human hepatoblastoma cell line transfected with HBV DNA) were used to investigate the molecular mechanism of IL-6/RT on HBV reactivation.

Results: Radiation induced HBV reactivation was in mice treated with combined IL-6 and RT (4.2-fold) but not with RT (1.0-fold, p=0.0004), IL-6 (1.1-fold, p=0.0004), or sham groups (1.0-fold, p=0.0003). Increased serum IL-6 simultaneously with RT was needed to reactivate HBV. HBV core protein staining confirmed the intrahepatic HBV reactivation. IL-6/RT induced HBV DNA replication in HepG2.2.15 cells, which was suppressed by STAT3 inhibitor AG490. Transfection with dominant-negative STAT3 plasmid showed the STAT3 pathway involved in HBV reactivation by IL-6/RT. Furthermore, the strongest staining of phospho-STAT3 was revealed in liver treated with combined IL-6/RT.

Conclusions: Radiation induced HBV reactivation in liver is by synergistic effect of IL-6 on RT through STAT3 signal transduction pathway.

YI-06

The prognostic values of expression of M-CSF and macrophages in peritumoral liver tissue after curative resection of hepatocellular carcinoma

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[1] Liver Cancer Institute and Zhongshan Hospital, Fudan University, Shanghai

Purpose: To investigate the prognostic values of the intratumoral and peritumoral macrophage colony-stimulating factors (M-CSF) and macrophages expression in hepatocellular carcinoma (HCC) patients after curative resection.

Patients and Methods: Density of M-CSF and macrophages (MΦ) was assessed by immunohistochemistry in tissue microarray containing paired tumor tissue and peritumoral liver tissue from 105 patients who received hepatectomy for histologically proved HCC. Prognostic effects of their densities and other clinicopathological factors were evaluated by Kaplan-Meier analysis and Cox regression, and their predictive effects were evaluated by ROC analysis.

Results: Neither intratumoral M-CSF nor MΦ density was associated with overall survival (OS) and disease-free survival (DFS). High peritumoral M-CSF and MΦ density, correlated with large tumor size, presence of intrahepatic metastasis and TNM stage, were independent prognostic factors for both poor OS ($P < 0.01$) and DFS ($P < 0.01$), and peritumoral M-CSF expression also affected incidence of early recurrence. In small HCC, only peritumoral M-CSF and HBeAg were correlated with both OS ($P = 0.038$, $P = 0.008$, respectively) and DFS ($P = 0.001$, $P = 0.015$, respectively). Combination of peritumoral M-CSF and MΦ had a better power to predict the patients' death (AUC = 0.751, $P < 0.001$) and recurrence (AUC = 0.731, $P < 0.001$).

Conclusion: Peritumorally high M-CSF and MΦ expression was associated with poor survival of HCC after hepatectomy, and may be useful marker for selection of more individualized post-operative adjuvant therapy. Peritumoral tissue in the recurrence and metastasis of HCC deserve further study.

YI-07

Coffee protects against liver fibrosis induced by DMN in SD rats

Jang Woo Shin[1], Jing Hua Wang [1], Chong Kwan Cho [1], Jong Koo Kang [2], Chang Gue Son [1]

[1] Daejeon University, [2] Chungbuk University

Coffee is one of the most highly consumed beverages worldwide, but thus far, coffee and human health studies have generally reported harmful effects. As the antifibrotic effects of coffee have been suggested only in epidemiological studies of liver cirrhosis, we sought to demonstrate these effects in experimental animals. Liver fibrosis was induced by injecting dimethylnitrosamine on three consecutive days weekly for 4 weeks. In the rats treated with coffee, we found that body weight, organ weight, and serum biochemistry improved significantly. Coffee markedly reduced hydroxyproline ($P < 0.001$) and malondialdehyde accumulation ($P < 0.05$) in the liver. Histopathological examination showed that necrosis/inflammation and fibrotic septa were significantly reduced in coffee-treated rats compared to control rats. Coffee inhibited the depletion of glutathione (GSH) and increased superoxide dismutase (SOD) in liver tissue. To investigate other molecular mechanisms of coffee, we closely analyzed gene expression in liver fibrosis. Coffee down-regulated mRNA expression for inducible nitric oxide synthase, transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and platelet-derived growth factor beta (PDGF-β) in the liver. *Conclusion*: In this study, coffee clearly showed protective effects against liver fibrosis, which originated from antioxidant properties that improved GSH and SOD and down-regulated pro-inflammatory cytokines such as TGF-β, TNF-α, IL-1, and PDGF-β. This is believed to be the first animal experiment demonstrating the effects of coffee on liver fibrosis.

YI-08

Serum type IV collagen: A marker to detect non-alcoholic steato hepatitis (NASH), among type II diabetes mellitus patients having non-alcoholic fatty liver disease (NAFLD).

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Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) covers a spectrum of liver diseases from simple fatty infiltration to progressive fibrosis. Non-Alcoholic Steato Hepatitis (NASH) can lead to cirrhosis and hepato cellularcarcinoma, both events that ultimately lead to early death. It is becoming the leading cause for referral to liver clinics in most areas. The prevalence of NAFLD in Indian population is estimated around 7 – 13%. The prevalence of NAFLD will likely continue to rise. Obesity, hyperglycemia, Type-II diabetes mellitus and hypertriglyceridemia are most important risk factors. In India high prevalence of Type-II diabetes mellitus is common. The prevalence of NAFLD is higher in type II diabetes mellitus.

Aim: To evaluate the Type-IV collagen – NASH test, a new bio marker for Non- Alcoholic Steato Hepatitis in patients with NAFLD.

Materials & Methods: 40 patients with NAFLD with out Type-II diabetes mellitus, 40 patients of NAFLD with Type-II diabetes mellitus and age & sex matched 47 normal healthy individuals as controls were selected for this study. Levels of serum Type-IV collagen, Electrophoretic separation of collagen, lipid profile and liver function test parameters were estimated in patients and compared to controls.

Results: Type – IV collagen levels were significantly increased in patients with NASH among the NAFLD patients compared to controls. Significantly higher levels of type-IV collagen are observed in patients with type-II diabetes mellitus when compared to the NAFLD patients without type-II diabetes mellitus. When compared to liver function test parameters and lipid profile levels, type-IV collagen has got positive negative predictive value among the NAFLD patients.

Conclusion: In patients with NAFLD, Type – IV collagen test, a simple and non – invasive and reliable to predict the presence or absence of NASH.

YI-09

Maximal endoscopic stenting for treatment of post-liver transplantation anastomotic biliary strictures

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Introduction: We investigated the effect of endoscopic retrograde cholangiopancreatography (ERCP) with maximal stenting on the outcome of post-orthotopic liver transplantation (OLT) anastomotic biliary strictures (AS).

Methods: Fifty-nine patients with post-OLT AS underwent ERCP with dilation and insertion of a maximal number of stents. Stents were not changed until signs or symptoms of obstruction were noted. Outcomes were AS resolution or hepaticojejunostomy (HJ). Four variables were evaluated: total number of stents, mean number of stents per ERCP, number of ERCPs, and time from OLT to AS diagnosis. Data were analyzed with t-tests.

Results: Median time from OLT to AS was 7 months. Median number of ERCPs was 3 (range 1-7). Fifty-five patients (93%) achieved resolution and 4 (7%) required HJ. Comparing the resolution and HJ groups, there were 8.3 (±5.4) (mean ±SD) vs 3.5 (±2.4) total stents ($p<0.016$), 2.5 (±1.2) vs 1.3 (±0. 47) stents per ERCP ($p=0.006$), 3.3 (±1.6) vs 2.5 (±1.3) ERCPs ($p=0.33$), and 14 (±19) vs. 22 (±42) months from OLT to AS diagnosis ($p=0.75$). One case of ERCP pancreatitis and none of cholangitis due to stent occlusion occurred out of 246 total ERCPs. In a median follow-up of 25 months, 2 patients had AS recurrence that was successfully re-treated with ERCP.

Conclusion: AS resolution is directly related to number of stents used in total and per ERCP. Maximal stenting for AS is effective, safe, seldom associated with AS recurrence, and conducive to less frequent ERCPs for stent exchange compared to conventional treatment.

YI-10

Intraductal cooling during radiofrequency ablation for periductal hepatocellular carcinoma: An evaluation of its safety and efficacy

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During the period between April 2001 and October 2006, 14 patients with periductal hepatocellular carcinoma (HCC), defined as tumours situated within 5mm from the central bile ducts, received central bile duct cooling during radiofrequency ablation (RFA) procedures in Queen Mary Hospital. RFA procedures for these patients with periductal HCC were performed by open surgery, with the initial removal of the gallbladder followed by cannulation of the common bile duct via the cystic duct for cold saline irrigation of the bile duct. The common bile duct was irrigated with 4ºC saline continuously during the RFA procedure using a pressure pump system, and the catheter was removed, with the cystic duct stump ligated, upon completion of the procedure. No hospital mortality resulted and only 2 patients suffered from minor complications including pleural effusion and ascites. Computed tomography scan was performed 1 month after the procedure and then every 3 months. All patients had complete ablation of the tumour after the RFA procedures and there was no local recurrence at the RFA treated sites. None of these patients developed liver failure, significant elevation of bilirubin level, clinical evidence of bile duct injury, or radiological evidence of any biliary complications during follow up. We concluded that intraoperative central bile duct cooling during RFA for periductal HCC is a safe and effective method for preventing biliary complications, without compromising the effectiveness of RFA in tumour ablation.

YI-11

The protective action of bicyclol against liver injury is mediated by its induction of heat shock proteins in mice

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Heat shock proteins (HSPs) are best known endogenous factors in protection against cell injury under various pathological conditions. Recently, emphasis is being placed on the potential use of these proteins in preventing and/or treating diseases. Therefore, it would be of great therapeutic benefit to discover compounds that are clinically safe and able to induce the accumulations of HSPs. Here we showed that a novel anti-hepatitis drug, bicyclol, induces HSP27/70 expression through activation of HSF1. The cytoprotective effect of HSP27/70 induced by bicyclol was observed in both acetaminophen (AP) and concanavalin A (ConA) caused liver injury, such as suppressed the elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), liver necrosis, the release of cytochrome C and apoptosis inducing factor (AIF) from mitochondria as well as hepatic DNA fragmentation, which consistent with the accumulation of HSP27/70. All the above actions of bicyclol against AP and ConA induced liver injury were attenuated by quercetin. Moreover, we also found that HSP27/70 appeared to inhibit NF-κB activation induced by ConA either by inhibiting IκB degradation or by directly repressing the NF-κB transcriptional activity. In addition, Overexpression of HSP27/70 induced by bicyclol suppressed ConA-induced activation of JNK signal transduction pathway. These results suggest that the protective action of bicyclol against liver injury is mediated by its

induction of HSP27/70, and provide new evidences for elucidating the mechanism of cytoprotective effect of bicyclol against liver injury both in animals and patients.

YI-12

Specific antibodies to alternate reading frame protein might influence the efficacy of antiviral treatment in hepatitis C patients

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The hepatitis C virus alternate reading frame protein (ARFP/F) of the 1b genotype is a double-frameshift product of the HCV core protein. We expressed recombinant F and core protein to investigate their clinical relevance, especially in relation to interferon treatment. An ELISA assay was developed using purified recombinant HCV core, F protein, as well as a synthetic F peptide. The prevalence of anti-F antibodies did not correlate with viral load, genotypes, or ALT level. The responding rate of the anti-F negative patients was 100% (15/15), while the responding rate of the anti-F positive patients was only 70% (40/57) before antiviral treatment (P=0.016). After interferon treatment, 24% of the responders (13/55) lost their anti-F antibodies, whereas no change in the antibody titer occurred for the 17 non-responders (p<0.001). HCV F protein elicits a specific antibody response. Specific antibodies to ARFP might influence the efficacy of antiviral treatment in hepatitis C patients.

YI-13

Close relationship between epithelial-mesenchymal transition and lung metastasis in hepatocellular carcinoma

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Purpose: To evaluate the relationship between epithelial mesenchymal transition (EMT) and lung metastasis in hepatocellular carcinoma.

Methods: there were 100 patients who underwent surgical resection of hepatocellular carcinoma between 2000 to 2004 in Liver Cancer Institute, Zhongshan Hospital, Fudan University. They were grouped with non-distance metastasis and lung metastasis according to the close following up till March 2007. Their hepatocellular carcinoma specimens were retrospectively examined for EMT markers(E-cadherin、Vimentin、Fibronectin) with immunochemistry staining in tissue microarray. Univariate and multivariate analysis were used for study the relationship between EMT and lung metastasis.

Results: Univariate analysis showed that lost membrane expression of E-cadherin, cytoplasm expression of vimentin, cytoplasm expression of fibronectin, AFP≥400ng/ml、tumor size more than 10cm, portal vein involvement had close correlation with lung metastasis. Multivariate analysis indicated that of cytoplasm expression of vimentin and fibronectin were independent factors for lung metastasis apart from AFP≥400ng/ml、tumor size more than 10cm and portal vein involvement.

Conclusion: The results proposed that EMT has close relation with metastasis in hepatocellular carcinoma.

YI-14

Eosinophils-involved in fulminant hepatic failure are associated with high IL-6 expression and absence of IL-5 in liver and peripheral blood

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Background/Aims: Although eosinophils are considered to play an important role in the pathogenesis of various parasitic, allergic and autoimmune digestive diseases, their role in fulminant hepatic failure (FHF) is unknown. Our contribution was to identify and quantify eosinophils and cytokine levels (interleukin-6, interleukin-5 and macrophage inflammatory protein-1α) in liver parenchyma and peripheral blood from FHF patients at pre- and post-transplantation steps.

Methods: Histochemical methods were used to identify/quantify eosinophils in liver samples. Liver and plasma cytokines levels were quantified using immunofluorescence methods. Results: FHF patients showed a high intrahepatic number of eosinophils concomitantly with an increased expression of IL-6, besides the IL-6 positive eosinophils associated with the lack of IL-5. It was accompanied by also raised number of eosinophils and soluble IL-6 and MIP-1α with poor expression of IL-5 in peripheral blood at the moment of liver transplant.

Conclusions: The increased intrahepatic number of eosinophils besides the high production of IL-6 may be involved in liver dysfunction. In addition, the poor presence of IL-5 in liver and peripheral blood may represent a particular pattern of eosinophil behavior in human liver failure, which may also involve MIP-1α. Further "ex vivo" studies are necessary to evaluate the specific role of eosinophils in FHF.

YI-15

Influences of hepatitis B virus basic core promoter on modulation of cycline-dependent kinase inhibitor p21WAF1/CIP1 expression and cell cycle progression in HBx -derived hepatocellular carcinoma：Vivo and vitro studies

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Hepatitis B virus (HBV) basic core promoter (BCP) located within HBV X gene (HBx) is suggested to be associated with hepatocellular carcinoma (HCC) development, and cyclin kinase inhibitor p21WAF1/CIP1 plays a crucial role in pathogenesis of malignant tumors. In present study, we investigated p21WAF1/CIP1 expression modulated by HBx depending on BCP and analyzed cell growth progression on carcinogenicity of HBV infected HCC. Detection of p21WAF1/CIP1 expression in hepatic tissues by immunohistochemistry and identification of BCP mutations in serum with PCR and sequencing were performed from 92 chronic HBV infection patients, including 32 cases of HCC, 28 of liver cirrhosis (LC) and 32 of chronic hepatitis B (CH), respectively. Plasmids of pCMV-HBx1 with BCP wild type and pCMV-HBx2 with BCP mutations were constructed. Four cell lines, HepG2, Huh7, Hep3B and NIH3T3 were transfected with pCMV-tag-HBx1, pCMV-tag-HBx2 and empty plasmid

pCMV-tag, respectively. p21WAF1/CIP1 expression was examined using western blotting. The effect of BCP on cell cycle progression was determined by flow-cytometric analysis. The positive rates of p21WAF1/CIP1 expression with BCP mutations, which showed as 21.7%, 21.8% and 41.7%, were significantly lower than that of 66.7%, 78.2% and 85.0% without BCP mutations for tissues of HCC, LC and CH, respectively. In HBx transfected cell lines, HBx1 enhanced p21WAF1/CIP1 expression and G0/G1 arrest was apparently demonstrated, while HBx2 strongly repressed p21WAF1/CIP1 expression and stimulated cell cycle at G1 to S checkpoint. These findings reveal that BCP mutations may be involved in carcinogenesis of HBx-mediated HCC by regulation of p21WAF1/CIP1 expression and cell cycle progression.

YI-16

Modeling viral dynamics and fitness of wild-type and resistant variants of HBV during treatment with a nucleotide analogue

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Background: Recently, treatment response to the nucleotide analogue Tenofovir disoproxil fumarate (TDF) was analyzed in patients developing genotypic resistance against lamivudine and also adefovir dipivoxil (ADV) in previous treatments. Even so cross-resistance of TDF and ADV has been reported, treatment with high-dose TDF showed a moderate and stable antiviral efficacy but also a selection of resistant variants. Here, we propose a general multi-variant model of viral kinetics.

Methods: We propose a differential equation system to describe the dynamics of wild-type as well as resistant variants with single and double mutants. The model uses different fitness parameters and treatment efficacies for each variant whereas further viral kinetic parameters such as viral clearance, infected-cell loss and de-novo infection rates are identical for all variants. The model also accounts for a shared and limited HBV replication space. It is used to fit HBV DNA kinetics and serial sequencing data in 10 patients treated with TDF.

Results: Overall, kinetics seems to be triphasic, a relatively fast first phase of viral decline during the first week, a slower but still declining phase and a flat phase when resistant variants became dominant. Only 2 of 10 patients had undetectable viral load after 12 months of treatment. Treatment efficacy factors and relative fitness of observed ADV resistant variants showed a high variation (50%-92% and 0.81-0.97, respectively) between different variants in each patient.

Conclusions: The multi-variant model can be successfully applied to describe the anti-viral activity of TDF and viral kinetics for treatment of ADV resistance.

YI-17

The variable impact of the metabolic component on ALT elevation in NAFLD patients based on the presence of central obesity

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is emerging as a major cause of liver disease that is associated with the metabolic syndrome including diabetes, hypertension and hyperlipidemia. The aim of this study was to determine the relative contribution of the different metabolic factors on alanine aminotransferase (ALT) activity in NAFLD based on the presence of central obesity.

Methods: A total 3070 patients diagnosed with fatty liver disease by ultrasound were enrolled. All components of the metabolic syndrome criteria, anthropometric parameters, routine biochemistry, the fasting plasma glucose (FPS) level, the insulin, high-sensitivity C-reactive protein (hs-CRP), and the lipid profile were measured.

Results: The prevalence of increased ALT levels (>40 IU/L) was 26.8%. Increased ALT activity was significantly associated with male gender, a young age, body mass index (BMI), waist circumference, diastolic blood pressure, fasting glucose, fasting insulin, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol and HOMA-IR. On the multiple regression analysis of the metabolic syndrome components, only triglyceride [odd ratio (OR) 1.859, 95% confidence interval (CI) 1.435-2.409, P<0.001], was an independent predictor of increased ALT activity without central obesity. However, in those with central obesity, triglyceride levels (OR 1.833, 95% CI 1.467-2.291, p<0.001), HDL-cholesterol levels (OR 1.474, 95% CI 1.040-2.091) and diastolic blood pressure (OR 1.409, 95% CI 1.128-1.760, p<0.001) were independent predictors of an increased ALT activity.

Conclusions: The impact of a metabolic component on ALT elevation can be different in NAFLD patients based on the presence of central obesity.

YI-18

Sensitivity and Specificity of Fibro Test in Patients with Chronic Hepatitis C/B at Different Stages of Hepatic Fibrosis

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Non-invasive diagnostics of hepatic fibrosis by Fibro Test aim to early and correct assessment of progression rate and control of stage of fibrosis during the therapy. Fibro Test includes 5 biochemical parameters combined in discriminant function: alpha 2-macroglobulin, gaptoglobin, apolipoprotein, g-glutamiltranspeptidase, the general bilirubin.

Estimation of sensitivity (Se) and specificity (Sp) of fibro test at patients with chronic hepatitis C/B at different stages of hepatic fibrosis (F1-F4 by Metavir score) was made.

We examined 50 pts with the main age 35±10 years. Thirty-three men and seventeen women were with BMI = 23.5±4.2 kg\m2. All patients were hospitalized in Vasilenko' Clinic due to chronic hepatitis – 84% (HCVRNA+) and 16% (HBVDNA+). Fibro Test and needle liver biopsy after Mengini were performed at the same day. The fibro test results were compared with the data of semiquantitative morphological analysis using Metavir score.

By the fibro test data Se was 70%, Sp – 85% for F1; Se - 80%, Sp – 100% for F2; Se - 100%, Sp – 100% for F3; Se – 100% and Sp – 100% for F4 stage of fibrosis. The Fibro Test data are highly authentic and informative, and the offered technique allows to perform non-invasive diagnostics of a degree of fibrosis liver at patients with chronic hepatitis C/B.

Thus, Fibro Test is represented as a new and prospective direction in early diagnostics of liver fibrosis at patients with chronic hepatitis C/B and used as alternative of Needle liver biopsy.

YI-19

The experimental study on a novel NASH mouse model accompanied with insulin resistance

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Non-alcoholic steatohepatitis (NASH), a character of metabolic syndrome, may progress to liver cirrhosis. The major aims of this study were to establish a novel NASH mouse model accompanied with insulin resistance (IR) and obesity, further to explore its molecular mechanisms and investigate the treatment

of NASH with PPARα agonist fenofibrate and PPARγ agonist rosiglitazone, respectively. The NASH model was induced by *ad libitum* feeding of the modified high-fat diet (mHFD) in C57BL/6 mice. As results, the models showed overt IR in insulin tolerant test. Compared with the controls, bodyweights of the models increased by 68%; plasma cholesterol, insulin and HOMA-IR index also elevated by 127%, 544% and 944%, respectively; The value of glucose infusion rate (GIR) in the hyperinsulinemic-euglycemic clamp was 7.2-fold lower. The models developed severe liver damages as indicated with serious steatosis, inflammation and fibrosis in morphological assessments, the hepatic triglyceride contents and serum ALT levels increased by 5 and 2 folds, respectively. The treatments of fenofibrate and rosiglitazone improved IR and dyslipidemia. The efficacy of fenofibrate was more effective in the treatment of NASH than rosiglitazone. In the mechanistic study, altered expressions of hepatic PPARα and PPARγ and their target genes led to enhanced lipogenesis and disturbed fatty acid β-oxidation. These results suggested that aberrant expressions of hepatic PPARα and PPARγ might play main roles in the pathogenesis of NASH via affecting lipogenesis and fatty acid oxidation in this novel NASH model.

YI-20

Bolstering hepatic cancer stem cells by Wnt/beta-catenin signaling

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Cancer progression/metastases and embryonic development share many properties including cellular plasticity, dynamic cell motility, and integral interaction with the microenvironment. Here, using global gene expression profiling, we identified a subtype of hepatocellular carcinoma (HCC) that resembled hepatic stem cell features. This subtype expressed alpha-fetoprotein (AFP) and a cell surface hepatic stem cell marker, EpCAM. A subset of $EpCAM^+$ AFP^+ HCC cells was identified by fluorescence-activated cell sorting as hepatic cancer stem cells with features for self-renewal and differentiation, and was highly invasive and could initiate metastatic HCC in NOD/SCID mice. Activation of Wnt/ -catenin signaling augmented the population of $EpCAM^+$ AFP^+ cells, while EpCAM blockage, a target of Wnt/ -catenin signaling, by RNA interference attenuated the activities of these cells. These $EpCAM^+$ AFP^+ HCC stem cell features are currently under investigation using clinical HCC specimens. Taken together, our results suggest that metastatic HCC growth and invasiveness is dictated by a subset of $EpCAM^+$ AFP^+ cells, which opens a new avenue for eradicating HCC cancer stem cells by targeting the Wnt/ -catenin signaling pathway including EpCAM.

YI-21

Deleted in liver cancer 1 negatively regulates Rho/ROCK/MLC pathway in hepatocellular carcinoma

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Liver cancer (hepatocellular carcinoma, HCC) is one of the most prevalent cancers worldwide. Intrahepatic metastasis is a major cause of mortality in HCC patients, and understanding the molecular mechanisms involved in intrahepatic metastasis is of crucial significance. Deleted in liver cancer 1 (DLC1), a tumor suppressor gene and a member of RhoGTPase activating protein (RhoGAP) family, possesses suppressive activities in tumorigenicity and cancer cell invasion in HCC. DLC1 has RhoGAP activity specific for inactivating RhoA. Rho-kinase (ROCK) is an immediate down-stream effector of RhoA in regulating cellular cytoskeletal events and cell movement. However, the underlying molecular mechanisms of how DLC1 represses cell motility have not been fully understood. In this study, we examined the effects of DLC1 on Rho/ROCK signaling pathway in hepatocellular carcinoma (HCC). We demonstrated that DLC1 antagonistically regulated ROCK-dependent actomyosin contractility. Ectopic expression of DLC1 abrogated Rho/ROCK-mediated cytoskeletal reorganization including formation of stress fibers and focal adhesions. DLC1 also suppressed cortical phosphorylation of myosin light chain 2 (MLC2). We demonstrated that these inhibitory events by DLC1 were RhoGAP-dependent, as RhoGAP-deficient mutant of DLC1 (DLC1 K714E) lost the above inhibitory functions of DLC1. Furthermore, DLC1 inhibited ROCK-related myosin light chain phosphatase targeting unit 1 (MYPT1) phosphorylation at Threonine 853. In addition, ectopic expression of dominant-active ROCK released cells from DLC1-induced cytoskeletal collapse and cell shrinkage. Our data suggest that DLC1 negatively regulates Rho/ROCK/MLC2 and this implicates a ROCK-mediated pathway of DLC1 in suppressing metastasis of HCC cells.

YI-22

Mechanism of Anti-Hepatitis B Virus Activities of Silver Nanoparticles

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Introduction: Silver nanoparticles could inhibit the in vitro production of HBV RNA and extracellular virions. The objective of the present study was to investigate the anti-HBV mechanism of silver nanoparticles.

Methods: The interactions between silver nanoparticles and HBV DNA were determined by gel mobility shift assay and absorption titration assay. Using the HepAD38 cell line as the in vitro infection model, the binding activities between HBV viral particles and silver nanoparticles were revealed by transmission electronic microscopy, and the binding affinity was further determined in a co-incubation model by real time PCR.

Results: Our results revealed that silver nanoparticles (Ag10Ns and Ag50Ns) were able to inhibit the formation of intracellular HBV RNA and reduce the extracellular HBV DNA formation of HepAD38 cells by over 50% compared to the vehicle control. Gel mobility shift assay indicated that Ag10Ns could bind to HBV dsDNA at 1:50 DNA to silver molar ratio, and the absorption titration assay revealed that silver nanoparticles had good binding affinity to HBV DNA with the binding constant (Kb) of $(8.8 \pm 1.0) \times 10^5$ dm^3mol^{-1}. Ag10Ns also showed good binding affinity to HBV virions, as only 54% and 12% unbounded viral particles (vs silver-nanoparticle-free control) were detected after co-incubation with coated Ag10Ns for 10 and 60 min, respectively.

YI-23

HIV and gp120 increase HCV replication through TGF-β1 upregulation

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Background/Aims: HIV co-infection increases HCV-related hepatic fibrosis progression, HCV persistence, and decreases response rates to interferon-based anti-HCV therapy. We explored the possibility that circulating HIV and/or its proteins contribute to HCV pathogenesis through engagement of extracellular co-receptors on hepatocytes by using HCV replicon and infectious JFH1 models.

Methods: Inactivated HIV and recombinant HIV proteins were incubated with HCV replicon (a genotype 1b full-length HCV RNA and co-expresses *Renilla* luciferase) or infectious HCV JFH1 cells. TGF-β1 levels were measured by using human TGF-beta 1 ELISA Kit. To verify the dependence of HIV's effects on HCV on gp120 binding to its cognate coreceptors, neutralizing antibody to CCR5 or CXCR4 were also tested.

Results: We found that inactivated HIV or gp120 increases HCV replication and enhances HCV-regulated TGF-®1 expression in both a replicon and an infectious model of HCV. HIV and gp120 enhancement on HCV replication is neutralized by antibodies to CCR5 or CXCR4. We found that human TGF-®1 also enhanced HCV replication. HIV's effect on HCV replication was blocked by a neutralizing antibody to TGF-®1, indicating that its effects on HCV replication are TGF-β1 dependent.

Conclusions: HIV and gp120 has a proviral effect on HCV replication that is dependent on co-receptor engagement. HIV and gp120 appear to promote HCV replication through upregulating TGF-β1 in HCV-infected hepatocytes. These results implicate an effect of circulating HIV on innate antiviral immunity to HCV, and suggest a novel mechanism by which HIV not only enhances HCV replication but also contributes to hepatic fibrosis progression.

YI-24

Identification of N-glycan of AFP by lectin affinity microarray

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Objective: Glycan plays important role in living organisms with its structural diversity. A simple, rapid and high-throughput manner involving the assignment of functional glycans is needed in the glycoproteomics study.

Methods: Integrated with the characteristics of lectin and flurescence technique. Lectin microarray allows sensitive observation of multiple lectin-glycan interactions. It could be used to identify the minim glycan structure differences of glycoproteins.

Results: 1mg/ml maybe the optimum spotting concentration of lectin. There is a linear relationship between the glycoprotein concentration and the fluorescence signal intensity.

Conclusion: Gel-slide is an ideal slide for lectin microarray. The lectin microarray allows the detection of the glycoproteins' concentration, gives the special patterns of the glycoproteins and differentiates the difference of similar glycoproteins. The developed lectin microarray can give out the "glycol-label" of many diseases and can be apply to the early diagnosis of diseases. The lectin microarray surely would contribute to the research of glycoproteomics associated with tumor and other diseases.

Free Paper Presentation – Acute Liver Failure

OL-001

Fulminant hepatic failure: from basic science to therapeutic intervention

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Fulminant hepatic failure is defined by the sudden onset of severe liver injury accompanied by hepatic encephalopathy in an individual who previously had no evidence of liver disease. This disease causes multi-organ failure and is associated with a high mortality. The most frequently recognised cause of fulminant or subfulminant hepatic failure is viral hepatitis. In China, there are increasing cases of severe acute on chronic hepatitis B (AOC) which eventually leads to hepatic liver failure. Data is now emerging to support the hypothesis that irrespective of the aetiology of fulminant hepatic failure, the host's immune response (including production of proinflamatory cytokines and mediators) contributes to microcirculatory disturbances that result in hypoxic injury and cell death (apoptosis/necrosis). Impairment of the scavenger function of the reticuloendothelial cell system further contributes to reduced hepatic blood flow and ischaemic necrosis. An increased understanding of the molecular pathogenesis of fulminant hepatic failure now enables new molecular therapeutic modalities to be tested. It is clear that, given the complexity of this multi-dimensional disorder, the challenge is to provide a rational basis for the treatment of patients with fulminant hepatic failure. This might include enhancement or suppression of immune responsiveness by manipulation of endogenous cytokine synthesis or by cytokine administration and, at the same time, would need to employ strategies to increase hepatic regeneration.

OL-002

Removal of asymmetric dimethylarginine (ADMA) during artificial liver support using fractionated plasma separation and adsorption (Prometheus®)

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The liver is the main organ metabolising the endogenous nitric oxide synthase (NOS) inhibitor ADMA (asymmetric dimethylarginine). Hemodialysis is not efficient in removing ADMA as it is highly protein bound. Prometheus® is an extracorporeal liver support system based upon the method of Fractionated Plasma Separation and Adsorption (FPSA). The aim of our study was to assess the efficiency of the Prometheus® system in reducing high ADMA levels in patients with liver failure.

We studied 8 patients with acute-on-chronic liver failure and concomitant renal failure. Average MELD score was 22 ± 7 and seven patients were from the ICU. Two consecutive sessions of Prometheus® therapy for each 5±1 hours were performed in all patients. ADMA and its stereoisomer SDMA were determined using mass spectrometry – gas chromatography. ADMA levels were correlated to MELD score (r_s=0.58; p<0.0001). Before Prometheus® was started, levels of ADMA and of SDMA were elevated (1,3 ± 0,5 µmol/l and 1,7 ± 0,4 µmol/l, respectively). During Prometheus® treatments, plasma levels of ADMA dropped by a mean 25% (p<0.0001) and SDMA levels by 22% (p<0.0001). However, there was a significant rebound of ADMA concentrations between the two therapy sessions (p<0.01).

This study shows for the first time that plasma levels of ADMA can be effectively lowered by an artificial liver support system. This might play a pathophysiological role in the clinical effects of these systems as ADMA has been shown to reduce cerebral and renal blood flow as well as cardiac output.

OL-003

Imbalanced intrahepatic cytokine expression of interferon gamma, tumor necrosis factor alpha, and interleukin-10 in patients with acute-on-chronic liver failure associated with hepatitis B virus infection

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Goals: This study attempts to determine expressions of intrahepatic proinflammatory and anti-inflammatory cytokines as well as their secreting immunocytes to evaluate their roles in the pathogenesis of acute-on-chronic liver failure (ACLF) in chronically HBV-infected patients.

Background: ACLF generally affects patients with established, compensated chronic liver diseases who develop an acute deterioration in liver function. In China, HBV-associated ACLF patients account for more than 80% of ACLF patients due to a high prevalence of chronic Hepatitis B Virus (HBV) infection. Clinical observation showed that the deterioration of this disease may correlate with host immune responses, but related underlying mechanism remains largely unknown.

Study: In situ expressions of interferon © (IFN-©), tumor necrosis factor ⟨ (TNF-⟨), interleukin-10 (IL-10) and their secreting CD4, CD8 T cells and Kupffer cells (KCs) were analyzed in the livers of patients with ACLF, chronic hepatitis B (CHB), and normal controls (NC) using immunohistochemistry.

Results: Intrahepatic proinflammatory IFN-© and TNF-⟨ expressions were markedly up-regulated in ACLF compared to CHB and NC. However, similar anti-inflammatory IL-10 expressions were observed in ACLF and CHB. IFN-© over-expression correlated significantly with increased CD4 and CD8 T-cell accumulation. TNF-⟨ up-regulation also correlated significantly with increased KCs.

Conclusions: The imbalanced expression of proinflammatory and anti-inflammatory cytokines and increased accumulation of CD4, CD8 T cells and KCs may contribute to immuno-pathogenesis in HBV-infected ACLF.

OL-004

Modulating fat metabolism protects mice from immune-mediated liver injury

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Immunity and metabolism are closely linked. The liver is the most important metabolic organ in the body. However, the interactions between hepatocytes and the immune system are poorly understood. In mice developing concanavalin A-induced hepatitis (CIH), we found extensive lipid accumulation in hepatocytes. Enzymes involved in fat synthesis such as stearoyl-CoA desaturase 1 (SCD1) and fatty acid synthetase (FAS) were upregulated, while the AMP-activated protein kinase (AMPK) pathway, which is responsible for fat oxidation, was suppressed. When we induced CIH in SCD1 deficient mice we found these mice to be highly resistant to CIH revealed by serum ALT levels and histology examination. The mechanisms of the protective effect of SCD1 deficiency might be attributed to the modulation of critical cytokines (TNFα, IFNγ) and signaling pathways (STAT1, NF-κB) in CIH pathogenesis. The production of leptin, a critical linker between energy metabolism and immunity, was suppressed in SCD1 deficient mice. Exogenous leptin replacement experiment indicated that leptin was, at least, partially involved in the protection of SCD1 deficiency against CIH. Moreover, restoration of AMPK in CIH by metformin reduced CIH severity. In conclusion, our study suggests that modulating fat metabolism in liver may represent a novel method for treating immune-related liver diseases.

OL-005

Safety and efficacy of molecular adsorption recirculating system in acute liver failure

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Aim: Molecular Absorbing Recerculating System (MARS) is an extracorporeal ALF support system method using albumin-enriched dyalisate to remove albumin-bound toxins. The aim of this study is to find the complications during the treatments and to purify the toxic substances.

Material and Methods: From 1999 we performed 2027 treatments with MARS. We treated 191 patients: 39 Fulminant Hepatic Failure, 16 Primary No Function, 21 Delayed Function, 94 acute on chronic liver failure, 7 after hepatic resection and 14 with intractable pruritus.

Results: In our experience we divided the following complications by acute on chronic liver failure and acute liver failure population. In

94 acute on chronic liver failure patients we observed 1.8% complications. Severe Hypotension, hemorrhage and infections by venous central catheter represented the principal complications that determined the treatment suspension. In 83 patients with acute liver failure we wanted to demonstrate as the MARS treatment not determined a worsening of neurological status and hemodynamic parameters. Only 18 patients did not show an improvement of these two aspects.
Conclusion: We think that MARS treatment is an extracorporeal hepatic assistance technique that can be applied with complete tolerance for very long periods of time without hemocoagulative and hemodynamic complications.

OL-006
Characterization of circulating and liver-infiltrating immunologically-competent cells in patients with acute-on-chronic liver failure associated with hepatitis B virus infection
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Background/Aims: The immunological alterations in patients with acute-on-chronic liver failure (ACLF) in relation to chronic hepatitis B virus (HBV) infection remain poorly understood. This study attempts to characterize the feature of immunologically competent cells (ICCs) in HBV-infected ACLF patients.
Methods: Circulating ICCs were examined in ACLF patients (n = 75), as well as in patients with chronic hepatitis B (CHB, n = 31), stable hepatitis B-related liver cirrhosis (LC, n = 36), and normal controls (NC, n = 30). Isolated and *in situ* ICCs were further analyzed from liver tissue of some subjects.
Results: Circulating numbers of total lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells and NK cells were obviously decreased in ACLF group compared with CHB and NC groups but rather than LC group. Importantly, these cells were significantly decreased in non-surviving ACLF patients compared with surviving ACLF patients. In comparison with NC, ACLF patients displayed higher ratios of liver-infiltrating $CD4^+$ T-cell and $CD8^+$ T-cell frequencies to their circulating counterparts, suggesting that compartmentalization of the ICCs from peripheral into liver may partly account for altered distribution of the ICCs in ACLF. Immunohistochemical analysis showed that intrahepatic $CD4^+$ T-cell, $CD8^+$ T-cell and NK-cell counts were significantly increased in ACLF group compared with other three groups, indicating a strong cellular immune response-mediated liver injury in ACLF.
Conclusions: The abnormal prevalence of circulating and intrahepatic ICCs possibly acts as a driving factor for the progressive development of HBV-infected ACLF.

OL-007
Epigallocatechin-3-gallate protects mice from concanavalin A-induced hepatitis through suppressing immune-mediated liver injury
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Epigallocatechin-3-gallate (EGCG) is the major active component of green tea. Increasing evidence has suggested that EGCG exhibits anti-inflammatory, anti-oxidant and immunosuppressive effects. In this study, we investigated the effect of EGCG on concanavalin A (ConA)-induced hepatitis (CIH) in mice, a model of immune-mediated liver injury in humans. We pretreated mice with EGCG before ConA injection, and then measured alanine aminotransferase (ALT) levels in plasma, inflammatory infiltration and hepatocyte apoptosis in liver. Potential therapeutic mechanisms were elucidated further by measuring several inflammatory mediators. Mice pretreated with EGCG exhibited much less increased ALT levels in plasma, reduced inflammatory infiltration and hepatocyte apoptosis in liver compared with control mice pretreated with vehicle solutions. We further investigated the mechanisms of the protective effects of EGCG. In EGCG-pretreated mice, we found abrogated tumour necrosis factor (TNF)-α and interferon (IFN)-γ at both protein levels in plasma and mRNA levels in liver. At the same time, the concentration of nitrite in plasma and inducible nitric oxide synthase production in liver were both down-regulated in these mice. Moreover, IFN-inducible protein-10 and macrophage inflammatory protein-1α expressions in liver were decreased significantly. Therefore, EGCG is capable of regulating immune-mediated liver injury *in vivo*. The protective effect depended on its suppressive effect on the production of important inflammatory mediators.

OL-008
The function of mixed-microencapsulated rat hepatocyte and testicular sertoli cell in vitro
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Objective: To investigate the sustainable effects of mixed microcapsules of hepatocytes and testicular sertoli cells on liver function.
Methods: Four groups of microencapsulation were prepared in this study. They were microencapsulated hepatocytes alone (group Ⅰ), microencapsulated sertoli cells alone (group Ⅱ), mixture of microencapsulated hepatocytes and microencapsulated sertoli cells (group Ⅲ), and mixed-microencapsulated hepatocytes and sertoli cells (group Ⅳ). Supernatant was collected regularly to detect the secretion of albumin and urea.
Results: After 96 hours encapsulation, round hepatocytes and sertoli cells were dispersed evenly in the microencapsulates, which is sphere with smooth surface. Albumin and urea in the supernatant of group II were undetectable. Compared to group Ⅰ, the levels of albumin and urea in the supernatant of group III and IV increased significantly ($F=217.56$, $P<0.001$; $F=232.72$, $P<0.001$ respectively). In addition, cells in group III and IV survived much longer than cells in group I. The levels of albumin and urea in group IV peaked at the seventh day with the level of 2.80±0.11mg/mL and 1.92±0.10μmol/L respectively, while in group I and III, the peaks were at the third day. Compared to group IV, the levels of albumin and urea in group III decreased significantly in the late phase of microencapsulation.
Conclusions: The microenvironment created by mixed hepatocytes and sertoli cells might be the best condition to keep longevity of hepatocytes, and maintain function and morphology of hepatocytes.

OL-009
Primary non function: hemodynamic parameters improvement with albumin dialysis
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Introduction: Patients with Acute Liver Failure show an aggravated hyperdynamic circulation. We evaluated potential changes in systemic haemodynamics induced by the Molecular Adsorbent Recirculating System (MARS) in a group of patients with Primary no Function (PNF).
Material and Methods: In Intensive Care Unite we treated 16 patients with PNF (6 female and 10 male) after liver transplantation with a mean age 47.8 (range 28-60). Continuous MARS treatment was carried on all patients with kit change every 8 hours for mean days 10(range 2-20). Veno-venous access (double-lumen catheter type) was used for blood supply. Blood flow rate was 150-250 mL/min, depending on the hemodynamic situation of the patient. Blood passed through an albumin non-permeable, high flux dialysis membrane. During MARS treatment the hemodynamic condition, using a series of parameters such as Heart Rate (HR), Mean Arterial Pressure (MAP), Cardiac Index (CI), Systemic Vascular Resistance Index (SVRI), Pulmonar Vascular Resistance Index (PVRI), were monitored before (base value), after one hour (T1), after three hours (T2) and at the end of treatment (T3). Neurological status was value through Glasgow Coma Score (GCS) .The Sequential Organ Failure Assessment score (SOFA) was used as predictive criteria.
Results: There was a progressive decrease in the positive inotropic (dobutamine, norephinefrine) and significant improvement of hemodynamic parameters such as MAP ($p<0.001$), CI ($p<0.001$) and SVRI ($p<0.002$). Nine patients alive: 5 to liver transplantation (LT), 4 patients without LT. Seven patients dead: 4 after LT while 3 before liver transplantation for Multi-Organ Failure.
CONCLUSIONS: The MARS device significantly improves the hemodynamic targets in acute liver failure that also determine patients survival (52%) with PNF awaiting a re-transplantation presumably by a difference in removal rate of certain vasoactive substances.

Free Paper Presentation – Alcoholic Liver Disease

OL-010

Diet may modify the effect of alcohol consumption on chronic liver diseases and liver cirrhosis mortality – the Lifestyle and Mortality (LIMOR) study

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Introduction: Some laboratory evidence suggested that fruit, vegetable carbohydrate and animal protein may modify the effect of alcohol on liver cirrhosis but few epidemiological studies have investigated such interaction.

Methods: This case-control study collected past lifestyle and demographic data from proxy informants (relatives) of the deceased (cases) and of living controls in all 4 death registries in Hong Kong in 1998. Causes of death were provided by Department of Health. This analysis included 339 deaths from chronic liver diseases or liver cirrhosis (ICD-9 571) and 13115 controls. The mortality odd ratios (ORs) due to alcohol consumption were calculated using logistic regression adjusting for demographic, lifestyle, and dietary intakes. Interaction between diet and alcohol was estimated by interaction terms and stratification analysis.

Results: Compared with never and light drinkers (ethanol intake <168g/week in men or <112g/week in women), moderate and heavy drinkers (ethanol intake >=168g/week in men or >=112g/week in women) were associated with higher mortality OR (95% CI) of 3.89(2.74-5.50). Dietary intakes (fruit, vegetable, meat, fish and soy products) interacted with alcohol on the mortality (P<0.001). Among moderate and heavy drinkers, lower intake (<4 times/week) of fruit, vegetable, meat, fish and soy products produced higher mortality ORs (95% CI) of 6.03(3.15-11.56), 43.98(4.06-476.84), 7.30(2.30-23.11), 7.42(2.27-24.28) and 4.24(2.92-6.17), respectively. The corresponding mortality ORs for frequent intake (>=4 times/week) of these items were 3.49(2.28-5.34), 3.76(2.62-5.39), 3.78(2.61-5.47), 3.73(2.58-5.38) and 2.45(0.92-6.50).

Conclusion: Frequent intake of fruit, vegetable, meat, fish and soy may lower the risk of alcohol drinking on chronic liver disease and cirrhosis mortality.

OL-011

Chitoshan ameliorates the severity of steatohepatitis induced by high fat diet in rats

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Objective: Currently, no agent has been conclusively demonstrated to prevent the progression of nonalcoholic steatohepatitis (NASH). Chitosan, a natural product derived from chitin, was thought possess hypocholesterolemic properties. The aim of this study was to evaluate the potential effects of chitosan on nutritional steatohepatitis in rats.

Methods: Rats fed with high fat diet for 4 weeks to develope NASH which was confirmed by liver biopsy, and then 4 weeks of chitosan was given. Serum chemistry and liver histology were assessed and steato-inflammatory mechanisms were studied.

Results: Chitosan significantly protect against high fat diet-induced hepatic steatohepatitis. This effect was associated with repressed serum levels of total protein, globulin, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, total cholesterol and low density lipoprotein. Chitosan elevated the serum levels of high density lipoprotein and the ratio of albumin to globulin. Further, the condition of increased TNF-α, lipoemia, hyperinsulinemia, hyperleptinemia and hypoadiponectin in NASH were significantly ameliorated by treating with chitosan.

Conclusion: Chitosan effectively attenuated the steatohepatitis induced by high fat diet. The therapeutic effect of chitosan on NASH may be through exerting influence on adipokines.

OL-012

The expression and significance of adipocytokines in human nonalcoholic steatohepatitis liver tissues

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Objective: To explore the role of adipocytokines such as leptin and its receptors, transforming growth factor (TGF) beta1 and tumor necrosis factor (TNF) alpha in the pathogenesis of NASH.

Methods: Fifty-nine needle liver biopsy tissues from NASH patients were divided into three groups G1~3, S1~3, and F1~3 according to the severity of inflammatory (G), fibrosis (S), and steatosis (F) respectively. The protein and/or mRNA levels of leptin (Ob) and leptin receptor (Ob-R), TGF-beta1 and TNF-alpha in liver tissues were qualitatively and semi-quantitatively examined using immunohistochemistry, in situ hybridization and image analysis.

Results: The expression of leptin in NASH liver tissues was commonly found in zones 3 near the steatotic hepatocytes, as well as in the perisinusoidal and periportal fibrosis areas. The associations of leptin expression level with steatosis grade and fibrosis stage were statistically significant (P<0.05). TGF-beta1 was expressed in the area where fibrosis was obvious, and was closely associated with the inflammation grade and the fibrosis stage (P<0.05, r =0.298 and 0.339). The expression and distribution of TGF-beta1 paralleled to α-SMA positive hepatic stellate cells. The cells positive with TNF-alpha was closely correlated with the inflammatory grade in NASH liver tissues (P<0.05).

Conclusion: TNF-alpha might be the pivotal trigger and promoter in the initiative and progressive processes of steatosis and inflammation in NASH. Up-regulated expression of TGF-β1 and leptin, activation and proliferation of HSC might be important molecular mechanisms in the fibrogenesis and progression of NASH.

OL-013

Effect of Jianpi Huoxue decoction on cytokine pathway in rat liver induced by lipopolysaccharide

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Aim: To evaluate the effect of Jianpi Huoxue decoction (JHD), previously demonstrated inhibition alcoholic liver injury [1, 2], on lipopolysaccharide (LPS)-induced cytokine pathway.

Methods: Rats were oral administrated with saline solution or JHD (9g crude drug /kg/d) for 3d and challenged with LPS 50ug/kg via tail vein for 1.5h [3]. To observe: serous tumor necrosis factor alpha (TNF-α) (ELISA); liver histological changes (HE staining), Kupffer cells (CD68 immunohistochemical staining); CD68, phosphorylated inhibit-κB (p-IκB), TNF-α protein (western-blot); CD14, toll-like receptor (TLR) 2, TLR4, TNF-α mRNA (real-time RT-PCR).

Results: JHD significantly inhibit: hepatocyte tumefaction, cytoplasm dilution, microvesicular change, CD68-positive staining (Fig 1); CD68, p-IκB, TNF-α protein (Fig 3); TLR2, TNF-α mRNA (Fig 4) ; serous TNF-α (Fig2) induced by LPS except TLR4 mRNA decreased and CD14 mRNA increased unremarkably (Fig 4).

Conclusion: JHD inhibit LPS-induced cytokine pathway (CD68, p-IκB, TLR2, TNF-α), which provide a probably mechanism of JHD anti-alcoholic liver injury.

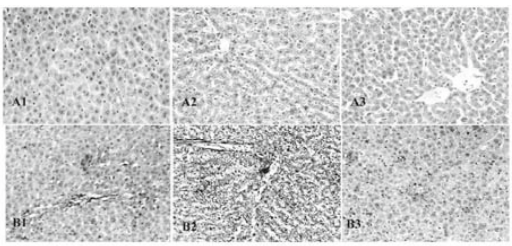

Fig.1 Effects of JHD on pathological changes and CD68 protein expression and distribution in liver tissue stimulated by LPS. A: H.E. staining X400. A1: liver tissue in normal group; A2: liver tissue in model group; A3: liver tissue in JHD group. B: CD68 Immunohistochemical staining X200. B1: liver tissue in normal group; B2: liver tissue in model group; B3: liver tissue in JHD group.

OL-014

Inhibition of adiponectin production by homocysteine: A potential mechanism for alcoholic liver disease

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Although recent evidence suggests that down-regulation of production of the adipocyte hormone adiponectin has pathophysiological consequences for the development of alcoholic liver disease (ALD), the underlying mechanisms are elusive. Abnormal hepatic methionine-homocysteine metabolism induced by prolonged alcohol exposure has been reported both in clinical and experimental studies of ALD. Here, we conducted both in vivo and in vitro experiments to examine the effects of prolonged alcohol exposure on homocysteine levels in adipose tissue, its potential involvement in regulating adiponectin production, and the consequences for ALD. Chronic alcohol exposure decreased the circulating adiponectin concentration and adiponectin messenger RNA (mRNA) and protein levels in epididymal fat pads. Alcohol feeding induced modest hyperhomocysteinemia and increased homocysteine levels in the epididymal fat pad, which was associated with decreased mRNA levels of cystationine beta-synthase. Betaine supplementation (1.5%, wt/vol) in the alcohol-fed mice reduced homocysteine accumulation in adipose tissue and improved adiponectin levels. Moreover, exogenous homocysteine administration reduced gene expression, protein levels, and secretion of adiponectin in primary adipocytes. Furthermore, rats fed a high-methionine diet (2%, wt/wt) were hyperhomocysteinemic and had decreased adiponectin levels in both plasma and adipose tissue, which was associated with suppressed AMP-activated protein kinase activation in the liver. Mechanistic studies revealed that both inactivation of the extracellular signal regulated kinase 1/2 pathway and induction of endoplasmic reticulum stress response, specifically C/EBP homologous protein expression, may contribute to the inhibitory effect exerted by homocysteine. Conclusion: Chronic alcohol feeding caused abnormal accumulation of homocysteine in adipocytes, which contributes to decreased adiponectin production in ALD.

OL-015

Effect of leptin on mouse model of alcoholic liver disease

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Aim: The purpose of this study was to evaluate the effect of leptin on inflammatory and profibrogenic responses in the liver of mice treated with hepatotoxic doses of ethanol.

Methods: CD-1 mice (n=15/group) were studied for 45 days. Four groups were studied: 1) control, 2) leptin+control (230 µg/kg intraperitoneal every alternate day from day 15), 3) alcohol (6.32 g/kg daily by gastric lavage, for 45 days) and 4) alcohol plus leptin (as prior dosing).

Results: Compared to controls, ethanol supplementation significantly increased levels of serum cytokines (TNF-a, IL-6, TGF-p<0.05, respectively) which was normalized by addition of Leptin (p<0.05). Reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS) in hepatic tissue were significantly increased and the anti-oxidant capacity as determined by superoxide dismutase (SOD) and glutathione (GSH), were significantly reduced by ethanol administration (p<0.05, respectively). These features were also normalised by addition of leptin. Moreover, mRNA levels were increased for 1 (I) procollagen (3 fold), MMP-2 (14 fold), MMP-9 (15 fold), TIMP-1 (0.5 fold) and Caspase-3 (1.6 fold) following ethanol administration as compared with control mice. Giving leptin to ethanol treated mice decreased mRNA expression of 1 (I) procollagen (1.5 fold), MMP-2 (3.2 fold), MMP-9 (3.5 fold), TIMP-1 (11 fold) and Caspase-3 (3.3 fold) as compared with ethanol alone fed mice. Liver histology showed that mice given ethanol had macrovesicular and microvesicular steatosis and apoptotic bodies. However, ethanol + leptin treated livers showed sinusoidal dilatation and no fatty change. Leptin injection in control mice showed mild sinusoidal dilatation and normal hepatocytes.

Conclusions: Thus our results suggest that leptin may protect against ethanol- induced inflammatory and profibrogenic responses in liver disease.

OL-016

Major investigative abnormalities, hepatic failure related complications and co morbid diseases in a cohort of adult Sri Lankan alcoholics admitted to a medical unit at the time of death

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R.L. Satarasinghe, P.A.K.G. Gunarathne, R.Ramesh, de Silva AP

Objectives: To evaluate the clinical profiles of major investigative abnormalities, associated hepatic failure related complications and major co morbid diseases in adult Sri Lankan alcoholics at the time of their death.

Design and setting: Case notes from death registry of 86 patients having proven alcoholic liver disease admitted to the principal author's unit at Sri Jayewardenepura General Hospital, Sri Lanka from 1.04.2002 to 31.12.2006 who had been endoscopicaly evaluated and fully investigated were retrospectively analyzed at the time death with respect to above.

Results: All were males with a mean age of 53.8±SD10.9 years with a range of 32 to 78 years. Major investigative abnormalities were as follows. Hb 9.1±SD2.4g/dl, RBS 170±SD98mg/dl, urea 74.5±SD64.4mg/dl (range 15-269), creatinine 210.7±SD150.4/µmol/l (range 75-1000/µmol/l), AST 148±SD388.9 IU/l (range 14-2970), ALT 69±SD120 IU/l (range 14-760), total bilirubin 6.2±SD6.9 mg/dl (range 0.1-26.2), albumin 2.7±SD0.7g/dl, INR 1.9±SD0.8 and APTT 53.5±SD18sec. Hepatic encephalopathy, upper gastrointestinal bleeding (UGIB), ascites, oesophageal varices, severe portal hypertensive gastropathy , spontaneous bacterial peritonitis and hepatorenal syndrome were present in 91%, 85.4%, 75.6%, 68.3%, 56.1%, 36.6% and 17.1% respectively. 65.8%, 17.1%, 17.1% were in grade C,B,A of Child Pugh classification respectively. Diabetes, hypertension and ischaemic heart disease were present in 75.6%, 26.8% and 2.4% of instances respectively.

Conclusions: Hepatic encephalopathy and UGIB were the most dominant complications. Diabetes was the commonest associated co morbid disease. Child Pugh grades were not reliable predictor of death in 1/3 of the cohort. Contribution from cardiovascular morbidity towards death was relatively less.

OL-017

Relationship between alcohol consumption and liver injury: A cohort study of alcohol-related liver injury in the island population from China

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Aims: To investigate alcohol-related liver injury in the island population from Zhejiang Province.

Methods: GGT, AST/ALT ratio and hepatic ultrasonography were regarded as the indicators of alcohol-related liver injury. 500 normal individuals in 1999 (excluding virus hepatitis) were followed up in 2006 (acquired 461 complete data).

Results: Logistic-regression analysis showed daily alcohol intake ≥ 40g, duration of drinking ≥ 10 years and body mass index (BMI) ≥ 25 kg/m^2 were related to liver injury, relative risk (RR) (95%CI) was 2.014 (1.108-3.662), 2.085 (1.106-3.928) and 1.772 (1.140-2.754), respectively (all *P*<0.05). Dose-response relation of alcohol consumption and liver injury was not apparent (Table 1).

Conclusions: Daily alcohol intake ≥ 40 g and duration of drinking ≥ 10 years were two important risk factors for alcohol-related liver injury in the island population. Alcoholic threshold effect rather than a dose-response effect on mortality from alcohol-related liver injury.

Relationship between alcohol consumption and liver injury: A cohort study of alcohol-related liver injury in the island population from China

Table 1 Dose-response relations between alcohol consumption and liver injury.

Characteristics	Total	A.L.I.	χ^2	*P*	RR(95%CI)
Daily alcohol intake (g)					
<40	282	49	–	–	–
40 ~ 80	61	21	8.976	0.003	1.981(1.289-3.045)
80 ~ 160	65	24	17.915	0.000	2.466(1.661-3.662)
≥160	62	26	17.982	0.000	2.413(1.637-3.558)
Duration of drinking (years)					
<10	217	31	–	–	–
10 ~ 20	46	19	17.995	0.000	2.891(1.799-4.646)
20 ~ 40	148	58	29.596	0.000	2.743(1.871-4.022)
≥40	50	12	2.838	0.092	1.680(0.930-3.034)

A.L.I.: alcohol-related liver injury

OL-018

Curcumin, a dietary natural phytochemical, ameliorates ethanol-induced cell injuries in rat primary hepatocytes via antioxidative approach

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Oxidative stress has been implicated in the pathogenesis of alcoholic liver disease (ALD). Curcumin, a dietary natural phytochemical, shows powerful antioxidant activity with cellular protection effects. However, whether curcumin treatment would ameliorate ethanol-induced hepatocytes oxidative damage has not been demonstrated and the underlying mechanisms are still unknown. Preliminary experiments were carried out in our laboratory to determine appropriate doses and durations for ethanol and/ or curcumin administration. In the present study, rat primary hepatocytes were isolated and divided into 6 groups: control, ethanol (100 mM), curcumin (5 μM) + ethanol (100 mM), curcumin (15 μM) + ethanol (100 mM), curcumin (30 μM) + ethanol (100 mM), curcumin (50 μM) + ethanol (100 mM). Enzyme releases in hepatocytes supernatant and oxidative/antioxidative status were detected. Microsomes of hepatocytes were extracted and heme oxygenase (HO-1) activities were determined. After the hepatocytes were treated with ethanol for 8 hours, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) releases and malondialdehyde (MDA) level increased significantly while glutathione (GSH), superoxide dismutase (SOD) levels and HO-1 activities decreased significantly. However, when the hepatocytes were pretreated with curcumin 1 hour before ethanol administration, significant attenuation of LDH, AST, MDA levels and elevation of GSH, SOD levels and HO-1 activities were observed, compared with the ethanol group. In conclusion, this study indicates that curcumin ameliorates ethanol-induced hepatocytes injuries via antioxidative approach, which suggests potential application in alcoholic liver disease prevention and treatment. HO-1 induction may be involved in the molecular mechanisms, however, further study is needed.

Free Paper Presentation – Fibrosis

OL-019

The Value of circulating and Intrahepatic Expression of Endoglin and Transforming Growth Factor- β in chronic hepatitis C Patients

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Endoglin (CD105) has been associated with renal and cutaneous fibrosis. Recent studies have reported the implication of CD105 in liver fibrogenesis. However, little information is available regarding serum or tissue expression of CD105 in patients with chronic hepatitis C virus (HCV) infection. Therefore, the aim of this study was to determine CD105 in serum and liver tissue of patients with chronic HCV infection and to correlate CD105 expression to stage of liver fibrosis and TGF-β1 and hyaluronic acid (HA). Forty-two chronic HCV patients and 20 healthy subjects were enrolled in this study. Western blot analysis was used to quantify endoglin and TGF-β1 in frozen liver biopsy samples, and serum concentrations of endoglin and hyaluronic acid (HA) were determined by ELISA. Our results revealed that intrahepatic expression of endoglin were significantly higher in severe fibrosis than moderate fibrosis ($p<0.05$), mild fibrosis ($p<0.01$) and normal liver ($p<0.001$). These findings correlated significantly with intrahepatic expression of TGF-β1. Additionally, the mean serum levels of endoglin were also significantly higher in severe fibrosis (7.9 ± 1.4 ng/mL) than moderate and mild fibrosis (5.4 ± 0.54 ng/mL, 3.6 ± 0.52 ng/mL, respectively, $p< 0.001$). Interestingly, serum endoglin showed a positive correlation with serum HA concentrations ($r=0.82$, $P<0.01$) and with the severity of hepatic fibrosis ($r=0.78$, $P<0.01$). In conclusion, increased serum and intrahepatic endoglin is significantly associated with progressive hepatic fibrosis in chronic HCV infection and the use of endoglin antagonist may be a fruitful therapeutic tool in hepatic fibrosis.

OL-020

Platelet-derived growth factor-BB induced activation of Rho-GTPases and actin cytoskeletal reorganization in rat hepatic stellate cells

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Background & Aims: In the liver fibrotic, the activated hepatic stellate cells (HSCs) undergo the shape reorganization and migration. Rho GTPases serve to be transducer signals between extracellular ligands and cytoskeleton reorganization. The aims of this study were to investigate the role of RhoA GTPase in the phenotype changes and migration of HSCs, and the intervention of PDGF-BB.

Methods: HSCs were transfected with wide type (wt) RhoA, constitutively active RhoA (Q63L) or dominant negative RhoA (T19N). A Transwell Chamber system was used to observe the changes of HSCs haptotactic and chemotactic migration after PDGF-BB treatment. Changes in actin cytoskeleton were visualized by fluorescence staining which recorded using confocal microscopy. The activation of GTP-loaded GTPases were evaluated by GST pull-down assays.

Results: PDGF-BB stimulated HSCs migration. Untreated cells had a rounded-up morphology. Stimulation of HSCs induced a rapid morphological change concomitant with a robust reorganization of actin cytoskeleton. GTP-bound Rac1, Cdc42 and RhoA GTPase activity was significantly augmented after PDGF-BB stimulation. Cells transfected with wide-type RhoA formed membrane ruffles and stress fibers at a normal ratio, while overexpression of constitutively active RhoA formed the enhancement of stress fibers and focal adhesion and the dominant negative mutant of RhoA caused the shrinkage cell shape and suppressed stress fiber formation. Both constitutively active RhoA and dominant negative RhoA inhibited cells migration.

Conclusions: The stimulation of PDGF-BB can induce the HSCs migration, which appears to be mediated through the activity of the Rho GTPases signaling pathway. RhoA regulate the formation of actin filament-based structures.

OL-021

Are simple non invasive tests useful to predict significant fibrosis in chronic hepatitis C patients?

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Aim: To determine how simple non invasive tests are useful to predict significant fibrosis.

Methods: A data of 323 CHC patients, who received a liver biopsy were studied retrospectively. Staging was performed according to Ishak. Patients were categorized as significant fibrosis (F3-F6), cirrhosis (F5-F6). AAR; APRI; GUCI; platelet count/spleen diameter ratio index and platelet count, has been proposed as an easily determined non invasive markers of liver fibrosis.

Results: Mean age of patients - 48.53 years, 39.9% females, 60.1% males. Non invasive markers were determined in 54.2% of patients with F3-F6, in 20.7% with F5-F6. Receiver operating characteristic curve analysis showed comparable diagnostic accuracies of AAR, APRI, GUCI, platelet count/spleen diameter ratio index and platelet count for prediction of F3-F6 [AUROC], 0.69, 0.65, 0.73, 0.74, and 0.71, respectively and for prediction of F5-F6 [AUROC], 0.85, 0.76, 0.89, 0.89, and 0.88, respectively. Diagnostic accuracy of APRI for prediction of F3-F6 was higher ($p<0.05$) to that of AAR, GUCI, platelet count/spleen diameter ratio index and platelet count. F3-F6 was reliably predicted by APRI>or=1.5, platelet count/spleen diameter ratio index<1.5, GUCI>1.5, AAR>or=1 and platelet count<150 x10(9)/L in 79.5%, 56.1%, 58%, 46.9% and 45.2% of the patients, respectively, whereas F5-F6 was reliably excluded by APRI<2.0, platelet count/spleen diameter ratio index>1.5, GUCI<2.0, AAR<1 and platelet count>or=150 x10(9)/L in 95.2%, 72.7%, 82.6%, 81.6% and 83.1%, respectively.

Conclusion: Simple non invasive liver fibrosis markers for CHC patients are useful in clinical settings when liver biopsy is unavailable (outpatient care, regional hospitals). However, these tests could not replace liver biopsy.

OL-022

Experimental study on anti-fibrosis effects of PGSL during hepatic fibrosis

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Objective: To further evaluate the anti-fibrosis effect of experienced Chinese herbal of PGSL by experiments.

Method: 53 SD rats were randomly divided into normal control group (n=8), fibrosis group (n=15), protective group (n=15) and treatment group (n=15). Immune hepatic fibrosis models were made by attacking rats with HSA. ALT was assayed using an automatic blood biochemistry analyzer. Contents of HA and LN were assessed by radioimmunoassay, and HYP content was measured using the method introduced by Shaoxiong Zheng. Plasma concentration of TGF-β_1 was assayed by ELISA. HE staining was performed to assess fibrosis proliferation and its distribution, proliferation extent of fibroblast, and alterations of hepatocytes and inflammatory cells. Type I and III collagen were respectively detected by VG and Argentophil staining [1]. α-SMA was observed by immunohistochemistry staining [2]. The spindle-shaped cells which existed at persinusoid location beyond portal and septa area were investigated by transmission electron microscope [3]. Hepatocytes were also observed by immune electron microscope.

Results: PGSL reduced the mortality, decreased levels of serum ALT ($P<0.01$), lightened the injury of hepatocytes, diminished contents of serum HA, LN, and liver HYP ($P<0.01$), depressed proliferation degrees of fibroblast, type I and III collagen, decreased serum TGF-β_1 level ($P<0.01$), reduced numbers of activated HSC ($P<0.01$) and depressed inflammatory cells infiltration.

Conclusion: PGSL could diminish mortality, protect hepatocytes, and delay as well as hinder fibrosis process.

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OL-023

CD45+ Collagen alpha1(I)-expressing cells (fibrocytes) possess high plasticity and mediate acute response to injury.

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Background: Bone marrow (BM) derived cells that express $CD45^+$ and type I collagen ($CD45^+Col^+$) are implicated in tissue healing and

repair. Using BM chimeric mice transplanted with collagen α1 (I)-GFP$^+$ BM we demonstrated that CD45$^+$Col$^+$ cells contribute to liver fibrosis induced by bile duct ligation (BDL) or CCl_4, comprising ≈5-10% of all collagen-producing cells. Moreover, liver injury stimulated homing of CD45$^+$Col$^+$ cells into the peripheral lymphoid tissues.

Aim: This study investigated the nature and function of CD45$^+$Col$^+$ cells.

Methods: To study CD45$^+$Col$^+$ cells we performed a series of adoptive transfer experiments, FACS analysis, transwell migration assay with TGF-β1 and LPS.

Results: Adoptively transferred CD45$^+$Col$^+$ cells migrated specifically to the injured liver but not lungs or kidneys of BDL mice. In contrast, transferred activated hepatic stellate cells did not migrate to the injured liver. Migration of CD45$^+$Col$^+$ cells was strongly induced *in vivo* and *in vitro* by TGF-β1 and another potent chemoattractant LPS. Surprisingly, 72 h after the injury, the majority of CD45$^+$Col$^+$ cells accumulated in lymphoid organs. We further examined the antigenic structure of splenic CD45$^+$Col$^+$ cells. Activated CD45$^+$Col$^+$ cells expressed CD11b^{hi} F4/80^{hi} Gr1^{low} MHCII^{med}CD83^{med}CD86^{hi} ICAM-1^{hi} B220^{hi}Sca-1^{med}CD48^{hi}CD1d^{hi} surface markers; CCR1, CCR2, CCR5, CXCR4 chemokine receptors; and secreted IL-12^{hi} IL-1^{med} TNF-α^{med} IFN-γ^{low} pro-inflammatory cytokines. In contrast, activated stellate cells stained positive only for ICAM-1^{hi}CD1d^{low} surface markers, indicating that these two types of cells differentiate from distinct lineages, and CD45$^+$Col$^+$ cells exhibit phenotypic characteristic of non-differentiated myeloid cells. Consistent with this, CD45$^+$Col$^+$ cells cultured in the presence of M-CSF (6 days) differentiated into macrophages (CD14$^+$F4/80$^+$CD11b$^+$), capable of *E. coli* phagocytosis. Activated stellate cells did not exhibit significant phagocytosis.

Conclusion: CD45$^+$Col$^+$ cells and hepatic stellate cells derive from distinct cellular lineages, exhibit distinct phenotypes and have different functions.

OL-024

Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of toll-like receptor 4 to hepatic stellate Cell responses

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We provide a mechanistic link that explains how specific TLR4 SNPs may confer risk of fibrosis progression. Here we show that two major alleles (wild type, WT) of TLR4 (Thr399 and Asp299) are related to higher clinical cirrhosis risk, while their common, highly co-segregating missense mutations (Thr399Ile and Asp299Gly) compromise hepatic stellate cell (HSC) responses and survival in vitro. Chronic hepatitis C patients that are WT homozygotes have 3 fold increased risk of developing bridging fibrosis/cirrhosis. TLR4$^{-/-}$ HSCs and LX-2 cells reconstituted with either TLR4 Asp299Gly and/or Thr399Ile cDNAs were hypo-responsive to LPS stimui compared to those with WT TLR4 cDNA, including LPS stimulated inflammatory & chemotactic cytokines (i.e., MCP-1, IL-6) expression and secretion, down-regulation of BAMBI (an inhibitory TGF-beta pseudoreceptor) expression, and activation of a NF-κB-responsive reporter. In addition, spontaneous apoptosis as well as apoptosis induction by pathway inhibitors of NF-κB, ERK, and PI3K were greatly increased in TLR4$^{-/-}$, MyD88$^{-/-}$ (an TLR adaptor protein), and TLR4 Asp299Gly and/or Thr399Ile expression mouse HSCs, correlated to decreased protein levels of phospho-ERK and Bcl-2. These finding demonstrate a critical role of TLR4 signaling in HSC biology and it is affected by TLR4 SNPs that are associated with fibrosis protection.

OL-025

Expression of cyclooxygenase-2 and 5-Lipoxygenase in human hepatic stellate cells

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Purpose: Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO) are two important enzymes that metabolize arachidonic acid (AA) and function in inflammation, tissue remodeling and fibrosis. The aim of the present study was to investigate the expression, function and inter-relationship of these two enzymes in human hepatic stellate cells (hu-HSCs).

Methods & Results: Primary isolated hu-HSCs were treated with NS398 (COX-2 inhibitor), AA-861 (5-LO inhibitor), and O5636 (dual inhibitor of both COX-2 and 5-LO) with or without 1μg/mlLPS administration.The induction of COX-2 and 5-LO proteins in hu-HSCs cells after LPS stimuli was detected by Western Bolt. Inhibition of COX-2 and 5-LO activities by their inhibitors in hu-HSCs cells decreased

LPS-induced inflammatory chemotactic cytokines (i.e.， MCP-1, IL-6) expression and compromised cell survival, as assessed by real-time quantitative PCR and MTT assay, espectively. Cells that were treated with COX-2 inhibitor (or 5-LO inhibitor) showed higher 5-LO (or COX-2) activity, as determined by specific EIA for COX-2 and 5-LO downstream products (i.e., PGE-2, TXB2 for COX-2, and LTB4 for 5-LO).

Conclusion: Both COX-2 and 5-LO were inducible in hu-HSCs under LPS stimuli. Complimentary effect of these two enzyme pathways in AA metabolism may exist and they are involved in the inflammatory phenotype of HSCs and promote cell survival.

OL-026

Doppler ultrasonography in the prediction of significant fibrosis in chronic hepatitis C patients

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Objective: The diagnostic value of Doppler ultra-sonography in the prediction of significant fibrosis in patients with chronic hepatitis C is unknown.

Patients and methods: The aim of the present study was to assess the prognostic value of Doppler ultra-sonography in the assessment of fibrosis in patients with CHC. 72 naïve-patients (M/F=45/27, mean age=46, range 24-62) with chronic hepatitis C and without evidence of cirrhosis were included. All patients underwent liver biopsy to asses degree of fibrosis (Metavir score), and Doppler ultrasonography. Doppler indices included portal vein velocity (PVV), hepatic arterial resistive index (HARI), hepatic arterial pulsatility index (HAPI), splenic arterial resistive index (SARI), and splenic arterial pulsatility index (SAPI). We also measured aspartate aminotransferase (AST) to platelet ratio index (APRI), age-platelet index (API), and AST to ALT ratio (AAR), for the diagnostic accuracy of significant hepatic fibrosis.

Results: In total significant fibrosis was observed in 49/72 (68%) patients. Among all the studied parameters SAPI index proved to be the more useful (P<0.001) in the prediction of significant fibrosis (≥ F2). When we compared the areas under the receiver operating characteristic for SAPI, API, AAR and APRI we found: 0.885 vs 677 vs 642 vs 511 respectively. SAPI value at 0.85 had sensitivity 97%, specificity 48%, positive predictive value (PPV) 45% and negative predictive value (NPV) 97% in predicting significant fibrosis.

Conclusions: The results of the present study showed that Doppler ultrasonography is a useful and powerful method in predicting significant fibrosis.

OL-027

Elevated plasma IL-4 level may enhance oxidative stress and development of complications in Egyptian patients affected with schistosomal liver disease.

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Some schistosomal patients may acquire infection and develop minimal complication while others may develop severe complications and progress to portal hypertension and cirrhosis especially if co-infected with hepatitis C virus (HCV). The reasons for this are poorly understood. This study aimed to investigate the status of the profibrotic IL-4 cytokine, oxidative stress (as indicated by thiobarbituric acid reactive substances), the antioxidants enzymes catalase and red blood cells glutathione content in Egyptian patients affected with pure schistosomal hepatic disease (SHF), mixed schistosomal and HCV infection and those with isolated HCV infection compared to an age and sex matched control subjects.

Results: A significant reduction in erythrocyte catalase activity in patients with isolated HCV infection, isolated SHF and those co-infected with SHF and HCV compared with the control group was found (P<0.05). A similar pattern was found regarding Erythrocyte glutathione content. Conversely TBARS level were significantly

increased in patients with HCV, SHF and mixed groups compared with the control group ($P<0.05$). Plasma IL-4 was significantly higher in patients with isolated SHF and those with SHF+HCV compared to the HCV alone patient group. Plasma IL-4 also correlated positively with portal vein diameter in SHF and SHF+HCV groups. ($r: 0.44$ & $P<0.05$).
Conclusion: Schsitosomal infection triggers a Th2 type immune response as indicated by high plasma IL-4. This may stimulate the production of reactive oxygen species with the resultant negative effects on antioxidants enzymes. These effects ultimately lead to disease progression and development of complications.

Free Paper Presentation – Liver Cancer

OL-028

The expression of human cytoskeletal protein septin4 is significantly reduced in HCC patients

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The septins are an evolutionarily conserved family of GTP-binding proteins which play an important role in a variety of cellular processes, such as cytokinesis, vesicle trafficking, apoptosis, and neoplasia. Septin proteins can form homo and hetero oligomeric polymers and can interact with some components of cytoskeleton. Recent studies have shown that alterations in expression profile of some of the family members are involved in neoplasia. For example, high expression of sept9-v1 is associated with oncogenic phenotypes in human breast cancer cells. Septin4 is also reported to have intimate relationship with tumorigenesis. An alternatively spliced form of Septin4 has been proved to be a pro-apoptotic protein and is lost in the majority of acute lymphoblastic leukemia patients. Here, we use quantitative real time PCR to detect the mRNA level of Septin4 in liver tumor tissue from 40 HCC patients, and found that 37 out of them had shown a reduced mRNA level in the tumor samples as compared with normal controls. These data support that the expression of Septin4 is significantly reduced in HCC patients. The abnormal expression pattern implies that Septin4 may play an important role in the pathogenesis of HCC, and further investigations are needed to illustrate the molecular mechanism in this progress.

OL-029

The prognostic significance of side population cells and their markers in hepatocellular carcinoma patients undergoing liver resection

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Background: In previous studies, we identified Side Population (SP) cells purified from Hepatocellular Carcinoma (HCC) cell lines with different metastatic potentials harbored similar CSCs-like properties and but different aggressive capacities. The goal of the current study is to elucidate the prognostic significance of SP cells and their markers in HCC patients underwent liver resection.

Methods: SP cells were sorted from 98 HCC patients underwent liver resection in our Institute between March 2007 and July 2007 by flow cytometry (FCM). CK19, CK7, CK10, ABCG2, CD133, Nestin, CD44, OV6, OPN and CD34, VEGF, PD-ECGF, which were putative biomarkers of SP cells (or Cancer Stem Cells, CSCs) and Vascular Niche (VN), were assessed by immunohistochemistry in tissue microarrays (TMA) containing 302 HCC patients underwent curative resection between 1997 and 2000. Prognostic effects were evaluated by Cox regression and Kaplan-Meier analysis. Predictive models were constructed based on Cox regression.

Results: HCC patients with high TNM stage, vascular/bile duct invasion or without encapsulation were prone to have high proportion of SP cells, which resulted in early recurrence as well as resistance to prophylactic TACE. All the biomarkers except CK7 were significantly related with overall survival (OS) and/or disease-free survival (DFS). The 5-year OS and DFS rates in CSCshigh/VNhigh group were significantly lower than those in CSCshigh/VNlow group, CSCslow/VNhigh group, or CSCslow/VNlow group, respectively. A simplified predictive model containing CD133, CD44, Nestin and MVD (microvessel density, determined by CD34 immunostaining) was an independent predictor for OS ($p<0.00001$) and DFS ($p<0.00001$).

Conclusions: Our results suggest that SP cells in HCC decide the aggressive potential beyond tumorigenesis. Co-expression of SP/CSCs and VN profiles simultaneously indicated very poor prognosis. SP cells and their markers may be novel prognostic indexes and therapeutic targets in HCC.

OL-030

Twenty-year survivors after ressection for hepatocellular carcinoma-analysis of 53 cases

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Objective: To clarity the clinicopathologic features of patients surviving ≥20 years after resection for hepatocellular carcinoma (HCC).

Methods: Between 1961 - 1987, 396 patients underwent hepatectomy for HCC; 53(13.4%) patients survived ≥20 years, and 343(86.6%) patients survived ＜20 years. A comparative study between the two groups was made.

Results: By March of 2007,67.6% (36/53) patients are still alive, disease free; 5.7% (3/53) patients died of tumor recurrence or metastasis; 11.3% (6/53) patients died of liver failure; 5.7% (3/53) patients died of other disease; 9.4% (5/53) patients were lost during follow-up. Reresection for recurrence was done in 9 patients, mean survival being 26 years and 11 months. Reresection for solitary pulmonary metastasis was carried out in 3 patients, mean survival being 29 years.

In comparison with patients surviving ＜20 years, patients surviving ≥20 years had higher asymptomatic tumors ($P < 0.01$), lower γ-glutany1 transpeptidase levels ($P<0.001$), lower incidence of liver cirrhosis ($P<0.01$), smaller tumors ($P<0.001$), higher percentage of single nodule tumor($P<0.001$) and well encapsulated tumors ($P<0.001$)，lower incidence of tumor emboli in portal vein ($P<0.01$), better differentiation of tumor cells ($P<0.05$), and higher curative resection rate ($P<0.001$).

Conclusions: Early and curative resection are the principal factors improving long-term survival. Long-term follow-up after resection of HCC is very important, and should continue for the remainder of the patient's life. Reresection for recurrence and metastasis is important approach to improve prognosis.

OL-031

Osteopontin expression in hepatocellular carcinoma (HCC) cell lines with different metastatic potential and HCC samples

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Objective: To study the relationships between OPN expression and HCC cell lines metastatic potential and HCC recurrence/metastasis after resection.

Methods: Immunocytochemistry, RT-PCR, Western Blot and ELISA were applied to detect OPN expression of HCC cell lines SMMC-7721, MHCC97-L and HCCLM6 with different metastatic potential. Immunohistochemistry of OPN was performed in the tissue arrays containing 200 HCC samples.

Result: We found that OPN expression was leveled with increasing metastatic potential of cell lines. OPN expression of samples was not associated with age, sex, serum AFP level, tumor size, cell differentiation, tumor capsule, lymphatic node metastasis while it was significantly associated with HCC TNM classification, portal vein tumor thrombi and HCC recurrence/metastasis after resection. OPN expression detected in 66% HCC samples of patients with recurrence/metastasis after resection was significantly higher than 37% samples of patients without recurrence /metastasis after resection ($P<0.05$).

Conclusion: OPN expression is associated with HCC cell line metastatic potential and maybe an indicator of HCC recurrence/metastasis after resection.

OL-032

Glypican- 3 amino terminal marker for early detection of hepatocellular carcinoma

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Introduction: Early detection of HCC in patients with liver cirrhosis goal for many years as HCC one of most prevalent cancer incidence still increasing because HCC develops from cirrhotic liver after chronic Infection hepatitis virus B or C. -AFP sensitivity not satisfactory especially in small HCC and complementary Serum marker clinically required .Glypican-3oncofetal protein over expressed in HCC

Aim: Evaluate Glypican-3an early detector of HCC

Methods: 20 patients with HCC defined by U.S and CT Liver function: (ALT, AST, BIL, Albumin GT)

Tumor marker: AFP, GPC-3

Viral marker: HCV antibodies and HBS antigen.

Results: AFP positive in 5% HCC and not correlated with GPC-3 GPC-3positive in 50% and false positive in 50% HCC documented by triphasic CT, positive in 50% of cases of liver cirrhosis. Diagnostic performance of AFP (cutoff 20) and Glypican -3 (cutoff 2000) showed AUROC of ***a*** AFPand GPC -3 where 0.632 in cirrhosis and spleenomegaly with 90 / ***%***CI (0.415 -0.848) and (0.407 -0.856) where ***a*** AFP showed 100%specificity and 55% sensitivity in cirrhoses with PPV 100% and 55% accuracy. Where GPC -3 showed 100% sensitivity, accuracy and PPV in cirrhosis.
Conclusion: GPC-***3*** proved to be early detector of HCC with 100%sensitivity and accuracy, able identify cirrhotic macrondules with malignant potential Glypican-***3*** Amino terminal marker for early detection of hepatocellular carcinoma**.**

OL-033
Lentiviral-mediated miRNA against oteopontin suppresses tumor growth and mtastasis of human hepatocellular carcinoma
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In our previous study, osteopontin (OPN) was identified as one of the leading genes that promote the metastasis of hepatocellular carcinoma (HCC). However, the mechanism by which OPN promotes metastasis of HCC is not understood yet. In this study, RNA interference (RNAi) mediated by viral vectors, which could induce a long-lasting down-regulation in gene expression, was applied to analyze the role of OPN in metastasis of HCC. Three lentiviral vectors encoding microRNA (miRNA) against OPN, Lenti.OPNi-1, -2 and -3, were constructed and found to down-regulate the OPN level by 62%, 78% and 95%, respectively, in HCCLM3 cells which had an over-expression of OPN and a higher metastatic potential. Consequently, both Lenti.OPNi-2 and Lenti.OPNi-3 induced a significant decrease in MMP-2 and uPA expression, and led to a obvious inhibition of both *in vitro* invasion and *in vivo* lung metastasis of HCCLM3 cells (P<0.001). Moreover, Len.OPNi-3, rather than Len.OPNi-2, could also suppress *in vitro* proliferation and *in vivo* tumor growth of HCCLM3. Smaller detectable tumors were found in only 50% of mice after implantation of Lenti.OPNi-3-transfected HCCLM3 cells (341 ± 502.6 mm^3 vs. >3500 mm^3 in controls, P<0.001). Len.OPNi-3, not Len.OPNi-2, significantly suppressed the mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK/ERK1/2) pathway in HCCLM3 cells. Recombinant OPN was found to induce translocation of NF-κB (p65) into the nucleus of HCC cell and the activation of MMP-2 and MEK/ERK/1/2.
Conclusion: OPN plays an important role in metastasis as well as tumor growth of HCC, in which different minimum threshold levels of OPN are needed. These effects might be through the activation of MAPK pathway and MMP-2. OPN could be a hopeful target for the control of HCC.

OL-034
Potential impact of macroscopic vascular invasion (MVI), ECOG performance status and extrahepatic spread (EHS) on sorafenib safety and efficacy in patients with advanced hepatocellular carcinoma: SHARP trial subset analyses
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Background: Macroscopic vascular invasion (MVI), extrahepatic spread (EHS) and ECOG performance status (PS) may impact sorafenib safety and efficacy in advanced HCC patients. We report 2 sub-analyses of stratification factors for the SHARP trial: ECOG PS (0 vs 1/2) and patients with/without MVI and/or EHS.
Methods: Patients with advanced, measurable HCC, ECOG PS 0–2, CP–A, received sorafenib (400mg bid) or placebo. Endpoints included overall survival (OS), time to progression (TTP), and safety. Radiologic assessment determined MVI presence.
Results: Efficacy data are tabulated below. No notable differences in the AE profile of sorafenib for PS 0 vs PS 1/2 patients were observed. Grade 3/4 drug-related AE incidence rates in the sorafenib arm for patients with/without MVI/EHS included diarrhea (5.3/15.7%), hand–foot skin reaction (6.7/10.1%), and fatigue (4.8/1.1%).
Conclusions: Regardless of ECOG PS or MVI/EHS presence, sorafenib extends survival in advanced HCC patients and is well-tolerated.

	Endpoint	Median months (sorafenib/placebo)	HR (95% CI)
Patients with MVI and/or EHS: sorafenib (n=209) vs placebo (n=212)	OS	8.9/6.7	0.77 (0.60, 0.99)
	TTP	4.1/2.7	0.64 (0.48, 0.84)
Patients without MVI and/or EHS: sorafenib (n=90) vs placebo (n=91)	OS	14.5/10.2	0.52 (0.32, 0.85)
	TTP	9.6/4.3	0.40 (0.23, 0.70)
Patients with PS 0: sorafenib (n=161) vs placebo (n=164)	OS	13.3/8.8	0.68 (0.50, 0.95)
	TTP	5.5/2.9	0.55 (0.40, 0.77)
Patients with PS 1-2: sorafenib (n=138) vs placebo (n=139)	OS	8.9/5.6	0.71 (0.52, 0.96)
	TTP	5.3/2.8	0.61 (0.42, 0.88)

OL-035
Gene signatures of distant metastases originated from primary solid tumors
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Metastases of solid tumors are the leading cause for cancer-related mortality, however current understanding of metastatic progression remains unclear. Previous gene expression profiling (GEP) studies of clinical specimens have indicated that some metastasis-associated genes can be found in primary tumors. To determine 'true' metastasis genes that are unique to metastatic lesions, we compared global GEP of primary solid tumors to their corresponding synchronous metastases using an Affymetrix U133 Plus 2.0 Array platform. Laser Capture Microdissection (LCM) was utilized to enrich tumor cells in resected samples that included eleven primary lesions of primary liver cancer (PLC) with synchronous lymph node, adrenal gland or lung metastases and 9 colorectal carcinoma specimens with synchronous liver metastases. An additional 10 metachronous lung metastases without primary Hepatocellular Carcinoma (HCC) lesions and 10 small HCC specimens without two year post-resection-relapse were also included. A comparison of primary and secondary tumors revealed virtually identical gene expression profiles at p<0.001 (cross validation). In contrast, when comparing PLC with or without metastases, we found 379 significant differentially expressed genes (p<0.001) in the lung metastases group (LM), 1085genes in the lymph node metastases group (LNM), and 1603 genes between non-metastasis PLC and liver metastases (GIM). Each metastasis-related gene set was fairly unique and consisted of only 12 overlapping genes among comparison groups. Our results suggest that genes favoring progression to metastasis are initiated in the primary tumors and interrogation of primary tumor lesions may allow for the advanced identification of cancer patients with a propensity to develop distinct metastases.

OL-036
Osteopontin is significantly expressed in advanced HCV-related liver disease and can accelerate HUH-7 cell growth in vitro and in a nude mouse model
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Osteopontin (OPN) is a multifunctional protein expressed in many tumours including HCC, however, the role of OPN in these disease processes is not well understood. The aim of this study was to

investigate the role of OPN in liver cell biology and its expression in chronic hepatitis C.
Results: RT-PCR revealed OPN is expressed as three distinct splice variants whose expression varied between different forms of liver disease. OPN expression increased with the severity of liver disease in chronic hepatitis C. Stable expression of these variants in Huh-7 cells resulted in OPN secretion and accelerated cell growth. Conditioned media from OPN expressing Huh-7 cells could stimulate growth of naïve Huh-7 and HepG2 cells that was blocked by addition of an anti-OPN Ab. Blocking CD44, a known OPN receptor also blocked proliferation. Subcutaneous injection of OPN expressing splice variant cell lines into nude-Balb/c mice resulted in significant tumor growth rates compared to non-expressing OPN controls.
Conclusion: We have demonstrated for the first time that OPN is significantly expressed as three distinct splice variants in HCV-related liver disease and HCC. In culture these variants are all secreted and can accelerate cellular proliferation in vitro and in an in vivo nude mouse model, via both autocrine and paracrine mechanisms. Increased expression of OPN in advanced stages of HCV-related liver disease suggests that OPN may play an important role in pre-neoplastic hepatocellular growth. Furthermore OPN may be a potential biomarker of advanced HCV-related liver disease as it is easily detectable in serum and urine.

OL-037
Differential proteins in serum between HCC and cirrhosis patients with HBV infection identified by cICAT-LC-MS/MS
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Background: Hepatocellular carcinoma (HCC) is one of the most common malignant liver tumor worldwide. The prognosis of HCC remains dismal mainly because of its late diagnosis especially in patients with cirrhosis background. Therefore more suitable serologic biomarker for HCC early diagnosis is urgently needed.
Methods: We depleted high abundant protein from serum, used a quantitative proteomics method based on cleavable ^{13}C-labeled ICAT reagents labeling combined with LC-MS/MS technology to identify potential serum biomarkers. Serum sample pools from HCC patients and liver cirrhosis with the hepatitis B virus (HBV) were analyzed and compared.
Results: A total of 96 proteins were identified and quantified, 22 displaying more than 1.5 fold expression differences between cirrhosis and HCC patients' serum. Isoform 1 of Haptoglobin-related protein precursor , Alpha-1-acid glycoprotein 1 precursor, Complement C4-A precursor, Prothrombin precursor (Fragment), Isoform 1 of Complement factor H precursor, Guanine deaminase, Isoform 1 of Probable ATP-dependent RNA helicase DHX40 were increased in HCC serum, whereas Alpha-2-macroglobulin precursor, Ubiquitin carboxyl-terminal hydrolase 44 decreased. Novel serum proteins associated with cirrhosis background HCC included Complement C4-A precursor, Isoform 1 of Complement factor H precursor, and Ubiquitin carboxyl-terminal hydrolase 44.
Conclusions: Diagnosis of HCC during early stages may be performed with a combination of AFP and these novel sera biomarkers, thus increasing the specificity and sensitivity for HCC early diagnosis. Further evaluation of these candidate markers needs to be performed in larger patient populations.

OL-038
Neuropilin-1 expression in peritumor predicts the favorable prognosis independent of vascular endothelial growth factor receptor-2 in hepatocellular carcinoma
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Objective: The reaction between Neuropilin-1 (NRP-1) and vascular endothelial growth factor (VEGF) is considered to be depended on VEGF receptor-2(VEGFR2). We sought to investigate the expression pattern of NRP-1 in peritumor and its prognostic significance compared with VEGFR2 in HCC.
Methods: NRP-1 and VEGFR2 expression were analyzed with 107 HCC tumor and peritumor tissue by immunohistochemistry based on tissue microarrays (TMAs). The clinicopathologic factors and patient survival were analyzed.
Results: NRP-1 and VEGFR2 expression (pixel) were both higher in peritumor compared with tumor, being 308787.75±207988.79 v. 726480.76±262573.14 (p=0.000); 308787.75±207988.79 v. 726480.76± 262573.14 (p=0.000). An univariate prognosis analysis showed that peritumoral NRP-1 and VEGFR2 were significant indicators for disease-free survival (DFS) respectively. (p=0.015; p=0.020).The combined analysis revealed that high NRP-1/low VEGFR2 group provided similar significant prognostic value as low NRP-1/ high VEGFR2 group and high NRP-1/high VEGFR2 group. Patients who had high peritumoral NRP-1 or (and) VEGFR2 levels had a markedly favorable prognosis. Multivariate analysis also demonstrated that the combined expression of NRP-1 and VEGFR2 was an independent prognostic indicator for DFS (RR=0.677, 95%CI: 0.473-0.969; p=0.033).
Conclusion: Higher expression of NRP-1 in peritumor of HCC could improve the DFS as well as VEGFR2 which may through their "decoy" effect for VEGF, and the signal transduction of VEGF/NRP-1 may be independent of VEGFR2. Combined expression pattern of NRP-1 and VEGFR2 provided more significant prognostic value for DFS.

OL-039
Metronomic chemotherapy with cyclophoshpamide and VEGF receptor inhibitor enhances treatment responses and survival in rat model of hepatocellular carcinoma.
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Conventional maximum tolerated dose (MTD) chemotherapy has been modest for survival benefit, but increased acute and chronic toxicity. Recent studies have demonstrated that frequent administrations of comparatively low dose of cytotoxic agents, with no extended breaks [low-dose metronomic (LDM) chemotherapy], may not only be as efficient as MTD therapy, but also less toxic. Therefore, we compared therapeutic effects of MTD cyclophosphamide (CTX), LDM-CTX and LDM-CTX plus a vascular endothelial growth factor receptor (VEGFR) inhibitor, bevacizumab, in hepatocellular carcinoma (HCC) rat model. Rats received weekly intraperitoneal (i.p) injections of diethylnitrosamine (DEN) at 50 mg/kg body weight in 16 weeks for induced HCC. HCC groups were divided into four groups: control HCC and LDM-CTX group received saline and 20 mg/kg CTX i.p injection thrice a week for 9 weeks, respectively. MTD group received 40 mg/kg CTX i.p injection on days 1, 3, and 5 of a 21-day cycle for 9 weeks. LDM-CTX plus bevacizumab group received 20 mg/kg CTX thrice a week and 5 mg/kg bevacizumb i.p injection every two weeks for 9 weeks, respectively. Growth modulating effects and overall survival of MTD-CTX, LDM-CTX and LDM-CTX plus bevacizumab were evaluated. LDM-CTX showed more significant reduction in tumor size than MTD-CTX. And, LDM-CTX plus bevacizumab had an additive tumor regression and showed more prolonged survival than MTD-CTX and LDM-CTX. In conclusion, LDM-CTX plus bevacizumab offers less toxicity over conventional MTD chemotherapy and would appear to be effective as combination therapy with targeted antiangiogenic agent in HCC.

OL-040
Transcatheter arterial chemoembolization for hepatocellular carcinoma with portal vein thrombi: long time survival and prognosis analysis
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Hepatocellular carcinoma with main trunk portal vein thrombi was difficulty problem for therapy. Transcatheter arterial chemoembolization has been used for treatment of HCC with main trunk portal vein thrombi and the efficacy and safety were controversial. From Dec 1994 to Dec 2006 there were 304 patients with hepatocellular carcinoma with portal vein thrombi treated with transcatheter arterial chemoembolization. Their age ranged from 18 to 80 years old (median 51 years old) with Child-Pugh class A in 272 patients and class B in 32 patients. There were 137 with main branch involvement and 167 with main trunk involvement. TACE was performed with intrahepatic artery infusion of 5-FU 1.0g, DDP 80mg and the oiled emulsion composed of lipoidol 5 ml to 20ml with MMC

20mg or epirubicin 50mg. Kaplan-Meier and Log-rank were used for evaluation of survival curve and survival difference. There were totally 584 TACE procedures performed (mean 1.98) in 304 patients. The severe complications were observed 5 of 584 TACE procedures (0.8%), including 4 with upper gastraduodenal bleeding and 1 with obstructive jaundice. There was 1 patient died of upper gastraduodenal bleeding (0.17%). The 1-, 2-, and 3-year survival was 38.7%, 19.4%, 15.0% respectively. There was no significant difference in survival for the patients with main branch or the patients with main trunk involvement (p=0.335). The significant prognostic factors were tumor diameter larger than 10cm (p=0.000) and alpha-fetoprotein level (p=0.000). Inclusion, TACE with chemotherapeutic agent infusion and oiled emulsion can be safely and seem to be effective for HCC with trunk or main branch portal vein thrombi.

OL-041

Fibroblast growth factor receptor 4 polymorphisms associated with the risk of hepatocellular carcinoma patients with portal vein pathological changes

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Purpose: Evaluated the association of fibroblast growth factor receptor 4 (FGFR4) with the risk of HCC development among patients in East China area with HCC and/or with HBV infection.

Methods: We genotyped the FGFR4 at four SNP sites in 2111 chinese persons, including 1012 patients with hepatocellular carcinoma, 595 individuals with HBV infection and 504 healthy subjects, using TaqMan SNP genotyping assay.

Results: For rs351855 (Arg388) locus, we observed a statistically significant that HCC patients with T/T homozygous genotypes had an inverse risk of tumor with gross portal vein tumor thrombosis (PVTT). For SNP rs641101, individuals with homozygous genotypes was a significant protective factor for HCC patients with portal hypertension (PHT).

Conclusions: Genetic polymorphism in FGFR4 is associated with the presence of HCC with portal vein pathological changes in Chinese patients.

OL-042

Autophagy defect is associated with malignant phenotype and poor prognosis of hepatocellular carcinoma in an apoptosis-deficient background

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Hepatocellular carcinoma is an aggressive cancer with a poor prognosis. The specific cellular malignant phenotype responsible for carcinogenesis and tumor progression in HCC is not well known. Here, we reported that HCC cell lines had decreased expression of autophagic genes, corresponding autophagic activity and increased expression of anti-apoptotic gene bcl-xL compared with normal liver cell line. The decreased expression of autophagic gene beclin 1 was further confirmed by 44 HCC tissues compared with adjacent non-tumor tissue, using real-time PCR and western blot analysis. Furthermore, the more aggressive malignant HCC cell lines and recurrent HCC tissues displayed much lower autophagic levels, especially when anti-apoptotic gene bcl-xL was overexpressed. Tissue microarray (TMA) of 300 HCC cases underwent curative resection between 1997 and 2000 were used to detect the expression of beclin 1 and bcl-xL by immunohistochemistry. In apoptosis impaired group (bcl-xL^+ group), the expression of beclin 1 was significantly correlated with DFS (P<0.0001) and OS (P<0.0001). Interestingly, in apoptosis competent group (bcl-xL^- group), there was no significant relationship between beclin 1 expression and survival (DFS and OS, P=0.978 and P=0.233). Beclin 1 was the strongest independent predictor in bcl-xL^+ patients for DFS and OS by both multivariate and univariate analysis. Moreover, we discovered that beclin 1 expression was correlated with tumor differentiation in bcl-xL^+ HCC patients (P=0.042)other than bcl-xL^- HCC patients(P=0.526). Conclusion: Autophagic genes expression and corresponding autophagic activity were suppressed in HCC. Autophagic defect determined the malignant phenotype and poor prognosis of HCC in an apoptosis-deficient background.

OL-043

TRP53 deletion induced liver carcinoma in mice containing stem/progenitor cells

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Background: In human the majority of hepatocellular carcinoma have defects in the p53-pathway. Due to the early onset of lymphoma and sarcoma in Trp53-/- mice the influence of the homozygous Trp53-deletion by itself on liver tumor formation could not be studied.

Methods: We used two mouse models with a liver specific Trp53 deletion by the expression of cre-recombinase to analyze the role of Trp53-deletion in hepatocarcinogenesis.

Results: We show that a liver specific deletion of Trp53 induces liver carcinoma in 12-14 month old mice reaching a tumor incidence of 100% in 18 month old mice. Histologically there was only small amount of classical precursor lesion, but oval cell aggregates and cholangioma appeared in 10-12 month old mice. In mice with a heterozygous deletion of Trp53 no liver tumor formation occurs. Trp53-/- liver carcinoma showed a mixed differentiation (hepatocellular and cholangiocellular). Tumor cell lines and primary tumors from liver-specific Trp53-/- mice exhibited tumor stem cell marker positive cells (CD133+, side population cells) but these cells did not exhibit enhanced tumorigenic potential compared to stem cell marker negative cells after transplantation in nude mice. In the context of chronic liver damage (HBs-transgene expression) Trp53-deletion induced both liver tumors of mixed differentiation (HCC/CCC) and classical HCC showing unilineage differentiation (hepatocellular). The level of chromosomal instability was lower in tumors with mixed differentiation compared to classical HCC.

Conclusions: Our studies in mice indicate that loss of Trp53 is sufficient to induce hepatocarcinogenesis. Moreover, deletion of Trp53 induces liver tumors exhibiting stem cell characteristics.

OL-044

Proteomics Discovery of AFP negative HCC Serum Biomarker with ClinProt

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CLINPROT™ System is an integrated set of tools for biomarker discovery and clinical proteomics research. We used this approach to identify new biomarkers of AFP negative HCC in the sera. Sera from 142 patients with cirrhosis which have been infected with HBV and their serum AFP level are all normal (≤20ng/ml), either without (n=64) or with (n=78) HCC, were purified with weak cation exchange (WCX) magnetic beads and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, autoflex®) to screen potential HCC markers. The spectra were analyzed statistically using flexAnalysis™ and ClinProt™ bioinformatic software. The highest-scoring peak cluster (receiver operative characteristic curves) was established in AFP negative HCC of serum samples (multinomial regression) and was tested in other independent groups, including AFP negative HCC, AFP positive HCC (AFP≥100ng/ml), cirrhosis and healthy controls. An algorithm including five highest-scoring peaks allowed correct classification (presence or absence of HCC) of 92.86% of patients in the test sample set and 91.67% in the validation sample set. For protein identification, WCX affinity bead–purified serum proteins from 90 patients were separated by tricine-SDS-PAGE and the gel slices according to the molecular weight of differential peaks were digested with trypsin. Proteins were identified by MALDI TOF/TOF MS and the MS together with MS/MS spectra were searched against the NCBI human database using the software GPS Explorer™ Version 3.0 and MASCOT database search algorithms (version 2.0). Three up-regulated proteins in HCC serum are cleavages of C-terminal parts of three proteins. Of them two were further validated by western blotting. In conclusion, global protein profiling is an efficient approach that enabled us to identify fragments of two new serum markers of AFP negative HCC.

OL-045

Neutralization of GEP enhanced hepatocellular carcinoma chemosensitivity to cisplatin

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Background and Aim: Hepatocellular carcinoma (HCC) is the second cause of cancer death in China. The granulin-epithelin precursor (GEP, also called progranulin, acrogranin, or PC-derived growth factor), a secreted glycoprotein, has been shown to be an important growth factor for HCC; suppression of GEP inhibited HCC cell growth *in vitro* and in nude mice xenograft model in our earlier studies. There are reports showing that GEP mediates drug resistance in some cancer types. Thus, we aim to examine if neutralization of GEP will sensitize HCC cells to chemotherapeutical drugs.

Methods: We examined the biological effect of GEP monoclonal antibody (mAb) A23, alone and in combination with cisplatin, on HCC cells (Hep3B) *in vitro*.

Results: Flow cytometry analysis of cell apoptosis revealed that Hep3B cells treated with GEP mAb A23 (100ug/ml) in combination with cisplatin (4ug/ml) demonstrated synergistic effect on induction of cell apoptosis (25.1%) compared to cisplatin alone (10.0%), GEP mAb A23 alone (0.1%) and control treatment (0%). PARP was examined to investigate if GEP mAb sensitized HCC cells to chemodrugs through activation of apoptosis pathways. Cleavage of PARP was detected in Hep3B cells treated with GEP mAb A23, cisplatin, and combination reagents, but not in control treatment.

Summary: The current data indicated that the GEP mAb A23 treatment enhanced the cytotoxic effect of cisplatin on HCC. The cell apoptotic event induced by cisplatin was amplified by neutralization of GEP, and the enhanced chemosensitivity was through activation of PARP apoptosis pathway.

OL-046

HBV Oncogenic Mutation Assay in Tumor and Paratumor Tissues from HCC Patients

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Objective: To identify the HBV oncogenic mutations related to hepatocellular carcinoma (HCC) in liver tumor tissue.

Method: HBV preS/S gene, X gene and precore region from 41 liver tumor tissues and 33 non-tumor tissues (among them 32 were paired) were analyzed by PCR direct sequencing.

Results: Phylogenetic analysis revealed that 87.3% strains were genotype C, 8% genotype B, 1.6% genotype D, 3.1% genotype A. X gene's carboxyl-terminal deletion (from 6 to 114 amino acids) was found in 52.6% (20/38) of tumor samples and in only 30% (9/30) of paratumor samples. Though without significant difference, the frequency of T1762/A1764 mutation and precore A1896 mutation were relatively higher in nontumor tissue: T1762/A1764 mutation or deletion in 75.6% (31/41) of tumor samples and in 86.7% (26/30) of paratumor samples; the A1896 mutation or deletion was detected in 64.9% (24/37) tumor samples and in 67.9% (19/28) of paratumor sample. Concordant to our previous work, pre-S1 start codon in-frame deletion, pre-S2 start codon mutation and pre-S2 in-frame deletion were all detected in both tumor tissue and paratumor tissue tested , with a higher tendency in tumor samples. Interestingly, pre-S1 deletion involving the S promoter was found in 5 tumor tissue while none in the paired paratumor tissues, indicating such HBV mutation may be liver tumor specificity.

Conclusion: Pre-S1 deletion involved S promoter sequence was probably oncogenic. The mutations involving pre-S2 gene, X gene, BCP, T1762/A1764 and precore A1896 were common in both tumor and paratumor tissues.

OL-047

Let-7g As A Potential Metastasis Suppressing Gene In Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is one of the most lethal cancers with dismal prognosis mainly due to metastasis and recurrence, and its incidence keeps rising in Western countries. MicroRNAs (miRNA) are a class of small, non-coding endogenous transcripts that regulate the expression of protein-coding genes at the translational level. miRNAs have also been implicated in various aspects of human disease, including cancer and its metastasis. Using the microarray technology, we have recently identified a signature of 20 miRNAs that can predict HCC patients with their propensity to metastasize. In this study, we determined whether let-7g, a specific miRNA found in the signature, is functionally linked to HCC metastasis. To validate the microarray results, we performed real-time RT-PCR analyses of let-7g on the original 22 HCC samples used in the array study. The results were further validated in an independent cohort of an additional 33 HCC cases. We found that patients with a low let-7g expression had significantly shorter overall and disease-free survivals when compared to cases with high let-7g expression. Forced expression of let-7g in HuH1 HCC cells resulted in a significant reduction in their ability to form colonies. Using a transwell migration and invasion assay, we found that let-7g-overexpressing HuH1 cells had a significant reduction in cell migration, but not invasion. The results were further confirmed by *in vitro* would-healing assay. Our results are consistent with the hypothesis that let-7g may act as a cancer suppressor and its reduced expression may confer metastasis by inhibiting HCC cell migration. We are currently investigating potential downstream targets of let-7g in HCC metastasis.

OL-048

Co-expression of putative biomarkers of cancer stem cells and vascular niche predict poor prognosis of hepatocellular carcinoma

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Background: To investigate the prognostic values of putative cancer stem cells (CSCs) and vascular niche (VN) biomarkers in hepatocellular carcinoma (HCC) patients.

Methods: Cytokeratin 19 (CK19), cytokeratin 7 (CK7), ABCG2, CD133, nestin, CD44, OV6, and CD34, vascular endothelial growth factor (VEGF), platelet derived endothelial cell growth factor (PD-ECGF), which were putative biomarkers of CSCs and VN, were assessed by immunohistochemistry in tissue microarrays (TMA) containing 302 HCC patients underwent curative resection between 1997 and 2000 in our institute. Prognostic effects were evaluated by Cox regression and Kaplan-Meier analysis. Predictive models were constructed based on Cox regression.

Findings: All the biomarkers except CK7 were significantly related with overall survival (OS) and/or disease-free survival (DFS). The 5-year OS and DFS rates were 79.5% and 81.3% for $CSCs^{low}/VN^{low}$ group, as compared with 55.2% and 50.5% for $CSCs^{high}/VN^{low}$ group, 56.4% and 41.0% for $CSCs^{low}/VN^{high}$ group, and 33.7% and 12.7% for $CSCs^{high}/VN^{high}$ group, respectively. A simplified model containing CD133, CD44, nestin and MVD (microvessel density, determined by CD34 immunostaining) was also an independent predictor for OS ($p<0.00001$) and DFS ($p<0.00001$).

Interpretation: Co-expression of CSCs and VN profiles simultaneously indicated very poor prognosis in HCC. The predictive model of CSCs and VN may help physicians make informed decisions regarding adjuvant treatments to HCC patients following curative resection.

OL-049

Down regulation of PTEN expression by Transforming growth factor -β1 through the Smad dependent and the Smad independent pathways

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Transforming growth factor (TGF)-β1 has been known as a tumor suppressor in epithelial cells, but TGF-β1 can also promote cellular invasion and tumor metastasis in advanced cancers. PTEN is a novel tumor suppressor with a dual-speciality phosphatase activity frequently mutated in various human cancers. It has been found that down-regulation of PTEN expression by TGF- β1 in human cancers may act as a tumor enhancer with specific effects on cellular motility and invasion，but the mechanism was still unknown. Here we confirmed that TGF- β1 can down-regulate PTEN protein and mRNA expression in human hepatocellular carcinoma SMMC-7721 cells. And we also found that down-regulation of pten expression was blocked by the the TβRI inhibitor SB431542, the MEK1 inhibitor PD98059 and the p38 inhibitor SB203580, which indicated that TGF- β1 down-regulate pten expression via multiple signaling pathways including Smad2, MEK1-ERK and p38. Further research will focus on whether TGF-β1 induced PTEN suppression results from the synergistic collaboration of the Smad2, MEK1-ERK and p38.

OL-050

New chemokine receptor RDC1 (CXCR7) involves in lung metastasis of human hepatocellular carcinoma

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RDC1 (CXCR7), once named orphan receptor, can respond to SDF-1 as CXCR4. To explore the effect of RDC1 in lung metastasis of HCC, expressions of RDC1 in human HCC cell lines with different lung metastasis potentials and the response of highly metastatic HCCLM3 to SDF-1 and lung extraction were examined. Further, tissue microarray was used to examine RDC1 in patients metastasized to lung after surgery. Effects of RDC1 on proliferation and metastasis of HCCLM3 cells in vitro and in athymic mice were evaluated by RNA interference. Effects of RDC1 on expressions of selected HCC metastasis-related genes were also evaluated. As a result, expression of RDC1 was significantly strong in HCCLM3 and elevated in companion with increased metastatic potential by gene chip, real-time PCR and Western blotting. HCCLM3 can respond to SDF-1 from 1μg/L to 100μg/L and lung extraction, which was not influenced by adding CXCR4 antibody. Tissue microarray showed patients with lung metastasis expressed RDC1 stronger than those without metastasis. Moreover, RDC1 expressed significantly stronger in tumor tissues, especially in diameter more than 5cm, than paracancerous tissues. Down-regulation of RDC1 in HCCLM3 decreased the ability of proliferation, invasion and metastasis of tumor cells, and expression of osteopontin, interleukin-8, and survivin. Down-regulation of RDC1 in HCCLM3 decreased the growth and lung metastasis of tumor cells in athymic mice. In conclusion, RDC1 contributes to the growth and lung metastasis of human HCC. RDC1 may be the potential molecular target of HCC metastatic therapy.

OL-051

Crucial role of side population cells in metastasis of human hepatocellular carcinoma

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Background: SP cells have been identified to endow with tumor-initiating capacity in several cell lines including HCC. However, their roles in metastasis and recurrence of HCC remain elusive. **Methods**: SP cells were sorted from 4 HCC cell lines with stepwise metastatic potential and 98 fresh HCC samples by flow cytometry, and then investigated by experiments of differentiation, clonogenic, MTT assay, western blotting, immunocytochemistry, qRT－PCR, matrigel invasive assay and in vivo tumor-imitating and metastasis assay. Finally, we evaluated the prognostic values of SP cells in 98 HCC patients using survival analysis.

Results: SP cells in HCC shared similar characteristics of self-renewal, high ABCG2, remarkable chemo-resistance, high clonogenicity and striking tumor-initiating abilities. The invasive ability and lung metastasis rate of SP cells in Hep3B, MHCC97-L, MHCC97-H and HCCLM3 increased stepwise in accordance with SP cell proportion of their parent cell lines. HCC patients with high TNM stage, vascular/bile duct invasion or without encapsulation were prone to have high SP cells, which resulted in early recurrence as well

OL-052

Pseudomonas aeruginosa vaccine inhibits the growth and metastasis of HCC through TNF-α and Fas/Fasl mediated apoptosis

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Objective: To investigate the effect and mechanism of pseudomonas aeruginosa vaccine (PA) on the growth and metastasis of hepatocellular carcinoma (HCC).

Methods: Nude (nu/nu) mice bearing orthotopic xenografts of human HCC were treated with PA given i.p at the dose of 0.1ml (5×10^{10} /ml) every day since the third day after tumor implantation. At the end of sixth week, the mice were killed and histopathological features, tumor volume, and lung metastasis were evaluated. Serum level of tumor necrosis factor (TNF)-α and activity of caspase 3, 8 and 9 of tumor samples were measured by ELISIA. Apoptosis and Fas/FasL expressions were evaluated by immunofluorescence and western blotting.

Results: Compared with control group, tumor growth was significantly inhibited ($3.12\pm0.85cm^3$ vs $0.79\pm0.36cm^3$, $P<0.05$) in mice treated with PA. The incidence of lung metastases of control and PA group were 66.7% and 0 ($P<0.01$) respectively. Tumor apoptosis of PA group was significant and the apoptosis index (AI) was 10.3±0.40 %, much higher than that of control group (1.4±0.37 %, $P<0.01$). Serum TNF-α was significantly upregulated by 50% than control group and activity of caspase 3, 8 and 9 were increased by 4.1, 2.3 and 1.9 fold respectively. Fas/FasL expressions were also elevated significantly in PA group.

Conclusion: These preclinical studies demonstrate that when given systemically, PA can induce the apoptosis of HCC by promoting the secretion of TNF-α and expression of Fas/FasL in nude mice, thus inhibit the growth and metastasis of HCC safely and effectively.

OL-053

Mechanism on the recruitment of EPCs into neovessels in adjacent non-tumorous tissues with hepatocellular carcinoma

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Purpose: We have first investigated the distribution, frequency and clinical significance of mobilized endothelial progenitor cells (EPCs) in hepatocellular carcinoma (HCC). However, why more EPCs were recruited into neovessels in adjacent non-tumor tissues (AT) is worth investigating further.

Experimental Design and Results: Based on immunohistochemistry on three tissue microarrays containing specimens from 128 patients with HCC (tumor tissues and consistent adjacent non-tumor tissues, TT and AT), the expression of Flk-1 and Tie-2 in HCC were higher in TT than AT, while VEGF, HIF-1α, CD105, MCP-1, COX-2, Angiopoientin-2, MMP-9, TGF-β, bFGF, endostatin, TIMP-2 and TSP-1 express stronger in AT, as determined by χ^2 tests. Consistently, among the 64 paired specimens, western blotting analysis revealed that the HIF-1alpha levels in AT was significantly higher than in TT. And ELISA analysis further revealed that the relative concentration of VEGF165 was lower in TT than AT. In addition, the scores of CD105, vascular cell adhesion molecule-1 and fibronectin staining were higher in AT relative to in TT. In cirrhostic mouse induced with CCl4, circulating endothelial progenitor cells, relevant with plasma VEGF and SDF-1, gradually increased during liver cirrhosis.

Conclusions: There were higher levels of HIF-1α, pro-angiogenic factors and cell-matrix adhesion molecules resulting from both liver cirrhosis and HCC, which recruited EPCs into AT.

OL-054

Expression significance and functional characterization of homeoprotein Six1 in hepatocellular carcinoma

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Homeoprotein Six1 plays an important role on regulation of metastasis in human cancers. The aim of this study was to unravel the role of Six1 in hepatocellular carcinoma (HCC) through clinical and experimental studies. Seventy-two pairs of RNA and 103 pairs of protein from tumor and adjacent nontumor liver tissues of HCC patients after hepatectomy were examined. About 85% and 60% of HCC tumor tissues overexpressed Six1 mRNA and protein, respectively, compared with nontumor liver tissues. No Six1 protein was detected in nontumor liver tissues of HCC patients and normal liver tissues. Increased Six1 protein expression in HCC patients was significantly correlated with pathologic tumor-node-metastasis (pTNM) stage, venous infiltration and poor overall survival. Short hairpin RNA interference approach was used to suppress the expression of Six1 in a metastatic HCC cell line MHCC97L. *In vitro* functional assays demonstrated that suppression of Six1 expression significantly suppressed the growth rate and proliferation ability of MHCC97L, and markedly decreased its motility and invasiveness. Data from xenograft tumorigenesis model demonstrated that *in vivo* growth rate of subcutaneous xenograft of MHCC97L was inhibited after suppression of Six1 expression. Experimental and spontaneous metastasis models indicated that suppression of Six1 noticeably reduced the pulmonary metastatic potential of MHCC97L. Our data suggested that Six1 is frequently overexpressed in HCC patients and elevated Six1 protein in HCC patients may be an indication of advanced stage and poor overall survival after hepatectomy. Suppression of Six1 leading to reduction of metastatic ability of metastatic cells implies its potential therapeutic application on treatment of HCC metastasis.

Free Paper Presentation – Viral Hepatitis (others) & Miscellaneous

OL-055

The effects of meteorology on onsets of esophagogastric variceal hemorrhage in patients with liver cirrhosis

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Objectives: The effects of meteorological factors on the onsets of Esophagogastric variceal hemorrhage (EVH) in patients with liver cirrhosis have not been known. The relationship between EVH onsets and the climate data was investigated.

Methods: A retrospective study was performed in 131 cirrhosis patients with EVH residing in Nanchang (a city in south-east of China) and hospitalizing in the First Affiliated Hospital of Nanchang University during 1999~2003. The daily meteorological data of Nanchang at the corresponding period were obtained from Jiangxi Observatory, and the daily air temperature, relative humidity and wind velocity were extracted from the data. The Human Comfort Index (HCI) were calculated according to the established formula.

Results: The onsets of EVH occurred in the Spring, Summer, Autumn and Winter were 26.0%, 15.6%, 25.9% and 31.9% (P=0.56), respectively. The EVH onsets negatively correlated with HCI and the average monthly atmospheric temperature (r=−0.6471, P<0.05; r=−0.6384, P<0.05), but positively correlated with the average standard deviation and coefficient of variation of monthly atmospheric temperature (r=0.7697, P<0.05; r=0.5620, P<0.05).

Conclusions: The onsets of EVH in liver cirrhosis patients are associated with meteorology: more frequent in Winter with low HCI, low atmospheric temperature and dramatic change of weather.

OL-056

Evaluation of liver histological lesions of atherosclerosis models in New Zealand white rabbits

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Aim: To evaluate liver histological lesions of atherosclerosis animal models induced by high lipid diet in New Zealand White rabbits.

Methods: Normal male New Zealand White rabbits (n=14) were randomly divided into two groups: control group (n=12) and atherosclerosis group (n=8). Atherosclerosis was induced by high lipid diet (92% normal rabbit chow with 2% cholesterol and 6% lard). Rabbits were sacrificed at the end of week 10. Serum aminotransferase**,** lipid levels and fasting plasma glucose (FPG) levels were examined dynamically and morphology changes in ascending aortas and livers were observed. The intima-to-media (I/M) ratio of ascending aortas was also measured.

Results: The levels of serum Alanine aminotransferase (ALT), total cholesterol (TC), triglyceride(TG), high density lipoprotein cholesterol (HDL), FPG in atherosclerosis group were significantly higher than those of control group (51.00±46.23 vs 21.33±17.76, 35.73±1.27 vs 0.69±0.22, 8.83±7.80 vs 0.56±0.10, 11.51±2.84 vs 0.42±0.14, 18.24±8.67 vs 7.47±2.47, *P*<0.05). Compared with control group, the livers presented the pathology of hepatic steatosis and steatohepatitis in atherosclerosis group, and ascending aortas showed typical atherosclerosis changes. The mean I/M ratio in ascending aortas of atherosclerosis group was higher than control group (*P*<0.05).

Conclusions: High lipid diet can induce an ideal rabbit model of atherosclerosis, and also induce severity liver histological lesion, and may be a right method to create the rabbit model in researching the relationship between fatty liver and atherosclerosis.

OL-057

CTLs induced by enhanced HDV epitope produce cross-genotype cytotoxicity

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Hepatitis D virus (HDV) quasispecies is an obstacle for developing effective vaccine. Whether the sequence changes in CD8 T cell epitope of HDV can make the virus escape from vaccine-induced immunity remains unknown. Peptides according to the sequence of twelve HDV isolates (including genotype 1, 2, and 4) derived from HLA-A2 patients were synthesized. Immunogenicity and cross-genotype immunity of HDV DNA vaccine and enhanced HDV epitopes were evaluated in HHD-2 mice. After immunization, cytotoxic T lymphocytes (CLTs) induced by HDV DNA vaccine (genotype 1) could lyse target cells only in the presence of high concentration of antigens, and limited cross-genotype CTL responses were observed. An enhanced HDV peptide (HDV43-51 3A; alanine substitution in position 3), being a better HLA-A2 binder, could generate powerful CTL response to wild type HDV. Ex vivo interferon-gamma ELISpot assay demonstrated that vaccination with the enhanced epitope produced high-avidity HDV-specific CTLs as compared with those produced by HDV DNA vaccine of wild-type sequence. In addition, CTLs induced by this modified epitope could generate significant cross-genotype cytotoxicity to most HDV variants of genotype 1, 2 and 3. In conclusion, CTLs induced by the enhanced HDV peptide can expand the range of T cell receptor (TCR) cross-reactivity and produce significant cross-genotype cytotoxicity. These findings may contribute to the design of HDV immunotherapy in the future.

OL-058

Metabolic syndrome can not predict histological nonalcoholic steatohepatitis (NASH) in patients with nonalcoholic fatty liver disease (NAFLD).

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Background/Aims: There is no effective non-invasive method to differentiate patients with or without histological NASH among patients with NAFLD. Aim of this study was to determine if presence of metabolic syndrome could be used as a predictor of histological NASH in patients with NAFLD.

Methods: Fifty eight patients with NAFLD (July 2001- Jan 2007) (Mean age 38.2±8.0 years, M: F=41:17) were histologically classified into Class I-IV as per Matteoni et al (Gastroenterology 1999: 116; 1413-19). Those having NASH (Class III&IV) were further graded and staged as per Brunt et al (Am J Gastroenterol 1999:94; 2467-74). Differences in age, gender, BMI, waist, waist-hip ratio, serum insulin, insulin resistance (HOMA-IR), liver enzymes (AST, ALT) and presence of metabolic syndrome were studied between those with and without histological NASH. Metabolic syndrome (≥3 criteria) was defined as per adult treatment panel (ATP) III criteria with modified waist for Asian patients.

Results: Twenty nine (50%) patients had class I or II (without histological NASH) disease and other 29 (50%) had class III or IV (histological NASH) disease. Overall metabolic syndrome was present in 25 (45.5%) patients with NAFLD. Fifteen (51.7%) patients in class I or II disease (without histological NASH) had metabolic syndrome in comparison to 10 (38.5%) patients in class III or IV (with histological NASH) (p=0.8). Other studied parameters were not different amongst two groups.

Conclusion: Presence of metabolic syndrome can not be used as a predictor of histological NASH in patients with NAFLD.

OL-059

Difficulties in diagnosing an adult case of Wilson's disease

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Wilson′s disease is one of the most commonest hereditary causes of unclear hepatopathy. We report a case of Wilson′s disease in an adult male. A 34-year old male patient with a history of hyperlipidemia was admitted with symptoms of abdominal pain and hematuria. While detailed findings did not reveal any urinary tract abnormality, abnormal liver function tests, ultrasonography and liver biopsy, pointed towards Non Alcoholic Fatty Liver Disease (NAFLD). Subsequently, the patient received treatments for NAFLD, which also included lifestyle related changes. On follow-up, NAFLD remained unresolved, with deterioration of liver histology from fibrosis stage 1 to stage 3. Biochemical tests revealed, decreased levels of serum ceruloplasmin (7mg/dl) and the 24 hour urinary excretion of copper was found to be (1550ml). However, only traces of copper were detected on liver biopsy. Suspicion of Wilson′s disease led to further mutation analysis, by Direct Sequencing. Presence of a heterogeneity in the patients ATP7B gene confirmed Wilson′s disease. In adults and even elderly patients with sign symptoms similar to NAFLD, the possibility of Wilson′s disease needs to be considered, investigated and ruled out, before the initiation of long-term therapy.

OL-060

Study of role of farnesoid X receptor in hepatocarcinoma cells

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Farnesoid X receptor (FXR), a memeber of nuclear receptor superfamily, acts as a ligand-activated transcription factor regulating the transcription action of numerous essential genes involved in bile acid and cholesterol homeostasis. FXR has bile acids as natural ligands and can modulate hepatic conversion of cholesterol to bile acids so its antagonists are viewed as the drug development target for cholesterol-lowering reagents. In addition to cholestasis diseases, FXR might be also a potential target to develop anticancer drugs for hepatocellular carcinoma (HCC) due to its ability to regulate bile acid level that is essential for liver functions. However, the role of FXR involving in the HCC progression is still unclear.

In this study, a liver cancer cell line, HEPG2, was transfected and overexpressing FXR, in which several anticancer compounds including marketed antiestrogen (tamoxifen), XIAP inhibitor (HRL-XI-2), FXR agonist (HRL-FR-T6), or an angiogenesis inhibitor (HRL-AI-3) developed in our lab., was treated respectively or combinatorially to evaluate their antiproliferation effects. The overexpression of FXR in HEPG2 cells seems to sensitize the antiproliferation effects of these compounds that showed better antiproliferation effects than they were treated in native HEPG2 cells.

Apart from the action on HCV replication and bile acid metabolism, FXR might exert particular functions in hepatocarcinoma cell proliferation so its modulators should be useful as the combinatorial treatment for HCC.

OL-061

Cr51-EDTA permeability test in ascitic cirrhotic patients with and without history of spontaneous bacterial peritonitis

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Background and Aims: Impaired intestinal permeability (IP) may be implicated in spontaneous bacterial peritonitis (SBP) pathogenesis in cirrhotics. Urine ^{51}Cr-EDTA is a standardized test for evaluating IP. Since ^{51}Cr-EDTA has a small molecular weight it can be found in peritoneal spillage in ascitcs. Aim of the study was to assess IP in cirrhotics.

Methods: 48 consecutive cirrhotic pts (16 for each Child class) were enrolled; 20 pts had ascites, 10 of those had also a history of previous SBP. We also enrolled 48 healthy subjects. In healthy subjects ^{51}Cr-EDTA was < 3 %. The presence of ^{51Cr}-EDTA in the ascites was also evaluated.

Results: 22 out of the 48 pts had an altered IP as described by ^{51}Cr-EDTA urine test *vs* 2 out of 48 controls (46% *vs* 4% p< 0.05). IP impairment followed progressing Child status: Child A 4/16; Child B 6/16; Child C 12/16. 12 out of 20 ascitic pts *vs* 10 out of 28 non-ascitic pts had an impaired IP (60% *vs* 36% p< 0.05). 8 out of 10 pts with ascites and SBP history had an impaired IP *vs* 6 out of the 12 ascitics without SBP history (80 % *vs* 50 %; p< 0.05). ^{51}Cr-EDTA was present in ascites samples from all ascitic pts with history of SBP *vs* 2 out of the 12 pts with ascites without SBP history (100% *vs* 22 %; p< 0.05).

Conclusions: a consistent number of cirrhotics have an altered IP. The presence of ^{51}Cr-EDTA in ascites in all pts with an history of SBP suggests an altered permeability of the splancnic vessels and/or peritoneal membranes.

Further studies are needed to assess a ^{51}Cr-EDTA urine and ascites cut-off where SBP profilatic therapy could be indicated.

OL-062

Molecular adsorbent recirculating system in patients with primary non-function and other causes of graft dysfunction after liver transplantation

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Introduction and Aim: Liver Dysfunction following liver transplantation (LTx) is an important cause of morbidity and mortality. It can present as Primary Non Function (PNF) or primary Graft Dysfunction (GD) and its prevalence is increasing because of the more frequent use of non-standard organs. The Molecular Adsorbent Recirculating System (MARS) is an albumin-based dialysis system designed to enhance the excretory function of a failing liver. Aim is to evaluate the potential role of MARS in this particular setting of patients. Primary end-point: six months survival.

Methods: all cases of Liver Dysfunction following LTx during the last five years were referred to our Intensive Care Unit. Patients presenting with progressively increasing jaundice (serum bilirubin level >15 mg/dl), and at least one of the following: hepatic encephalopathy, renal dysfunction, and intractable pruritus were treated with MARS in addition to standard medical therapy and included in this retrospective study.

Results: Seven patients median age of 52 years, with primary non function (2 cases) and graft dysfunction (5 cases) after LTx, were included in the study. Five patients had received a non-standard organ. Six-month overall survival was 71%; 5 cases of GD (1 re-LTx) were alive at the end of the follow-up, 2 cases of PNF (1 re-LTx) died. During MARS therapy in all patients there was a significant decrease in serum bilirubin level, bile acids, ammonia and creatinine levels. A sustained improvement of synthetic liver, neurological and renal functions were observed only in patients with graft dysfunction. Furthermore in 2 patients an improvement of the pruritus was observed.

Conclusions: MARS therapy is a promising and safe therapeutic option to treat severe GD after LTx.

OL-063

Post partum thyroiditis in women with chronic viral hepatitis

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Objectives: Post partum thyroiditis (PPT) is the occurrence of transient hyperthyroidism and/or transient hypothyroidism during the first year after delivery. The mean prevalence of PPT is 5-7.5%. Autoimmune thyroid disease represents a well-known extrahepatic manifestation in patients with chronic hepatitis C (CHC). The incidence of PPT among women with CHC has not been investigated yet.

Methods: During 2006, 21 women with CHC and 74 with chronic hepatitis B (CHB) had delivery in our hospital. All of them were prospectively evaluated for the appearance of PPT, defined as clinical hyperthyroidism or hypothyroidism and/or abnormal FT4/TSH levels with positive anti-TPO titers, during the first year postpartum.

Results: Five of 21 women with CHC developed PPT (23.8%), a percentage higher enough than the ones reported in the literature. None of 74 women with CHB developed PPT. Two women exhibited overt hyperthyroidism during the third and the sixth month postpartum, respectively, clinically (fatigue, tachycardia, sleep disorders, mild depression) and laboratory (suppressed TSH levels<0.02 mIU/ml and high anti-TPO titers) confirmed. The rest three women who developed PPT were presented with hypothyroidism (TSH levels 6.5, 18 and 61 mIU/ml, respectively as well as high anti-TPO titers).

Conclusion: Our findings are suggestive of a possible new high risk group for PPT, women with chronic hepatitis C, in whom screening might be beneficial. Prospectively designed, controlled studies, with large numbers of chronic HCV infected pregnant women are needed in order to clarify a possible relationship between chronic HCV infection and PPT.

OL-064

The course of chronic infection with hepatitis Delta virus: a 20-year cohort study of 299 Italian patients.

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Background: chronic infection with HDV was originally described as a severe disease, often leading to cirrhosis and liver failure.

Aim: to evaluate the evolutionary course of patients with HDV-related chronic liver disease, consecutively observed between 1978 and 2006.

Patients and methods: 299 patients (230 males, mean age 30yrs) with chronic hepatitis D. Modality of infection was unknown in 222 (74%), transfusion-related in 27 (9%), intravenous drug use in 38 (13%), sexual in 12 (4%). A liver biopsy was obtained in 251 (84%): AVH

was diagnosed in 7 (3%), mild-moderate CH in 101 (40%), severe CH in 76 (30%), and cirrhosis in 67 (27%). 272 (91%) were HBeAg negative and 59/261 (23%) were anti-HCV positive. 90 patients (30%) were treated with IFN, 62 (21%) with corticosteroids, and 12 (4%) with nucleoside analogues. 135 (45%) received no therapy. HDV infection was defined by serum IgM anti-HDV and/or HDV antigen in liver biopsies.

Results: After a mean follow-up of 208 months, cirrhosis were 181 (a.i. 10.34%). 46 developed HCC (a.i. 2.8%), 43 ascites (a.i. 2.7%), 44 jaundice (a.i. 2.7%) and one encephalopathy (a.i 0.06%).142 patients (47%) are alive, 102 (34%) were lost to follow-up, and 55 (18%, 1.1% per year) died. Causes of death were liver failure in 33 (60%), non liver related malignancies in 3, HCC in 6, other in 13.

Conclusion: our study indicates that chronic hepatitis Delta is a long lasting disease with low incidence of major liver-related complications. Mortality for hepatic causes may be less than previously suggested.

OL-065

Descriptive study on characteristics of hepatic involvement in epidemic and non epidemic dengue infections in adult Sri –Lankan; Experience from a tertiary referral Center

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Objectives: To study the clinical pattern and natural history of hepatic involvement with other related clinically important matters during epidemic and non epidemic dengue infections affecting adult Sri Lankans.

Design and Setting: Clinical notes of two age and sex matched cohorts of adult Sri Lankans comprising 219 and 220 patients admitted to the principal authors unit at Sri Jayewardene General Hospital – Kotte Sri Lanka from 1.06.2002 to 1.06.2003(epidemic) and 30.06.04 to 30.06.05 (non epidemic) and again from 01.11.06 to 01.11.07 (non epidemic) were respectively analyzed with regard to demographics, other clinically relevant abnormalities and were followed to date.

Results: Epidemic cohort: age distribution 12-65 years with a sex distribution of male: female 2:1. Hepatic involvement was seen in 83% as asymptomatic elevation of transaminases: SGPT 178.8 ± SD 221.78 IU/L (range 13-2000), SGOT 213.9 ± SD 360.6 IU/L (range 28-3600). Myositis (elevated CPK) was seen in 32%. Myocarditis (2D-Echocardiographic abnormalities) was seen in 24%. The above for non epidemic cohort were; age12-65 yrs; male: female: 1.5; hepatic involvement in 80%, SGPT 168.9 ± SD 226.7 IU/L(range11-1855), SGOT 213.9 ± SD 360.6I U/L (range16- 3500);myositis in 35% and myocarditis in 8.6%. In both cohorts other liver functions were normal. Fulminant liver failure was absent. Hepatitis showed no relationship to blood groups, myositis, myocarditis, or haematological indices. Transaminases became normal in all within four weeks.

Conclusions: Benign asymptomatic anicteric hepatitis occurs in about 80% of both epidemic and non epidemic dengue patients. No predictors for dengue hepatitis were observed.

OL-066

Hepatitis C virus and ethanol induce aneuploidy by different intracellular pathways.

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Hepatocellular carcinoma (HCC) is a common cancer characterized by several etiologic factors; among these hepatitis C virus (HCV) and ethanol, alone or in association, play an important role in HCC development and progression. During progression of HCC, hepatic cells accumulate several chromosomal alterations, including aneuploidy. Aneuploidy often occurs as a result of mitosis dysregulation. Although, HCV proteins and ethanol are directly involved in induction of aneuploidy in HCC, the mechanism remains not well defined. We have already reported the ability of HCV core protein to lead mitotic arrest depending on unconventional activation of PKR. Thus, here we investigated, in detail, mitosis dysregulation in HepG2 polyclonal cells stably expressing all HCV proteins or HCV core protein alone. In addition, we analyzed mitosis dysregulation in HCV polyclonal cells treated with ethanol. Our results indicate that HCV proteins (especially core) cause a mitosis dysregulation by a delay in mitotic exit and altering expression of spindle-associated molecules (i.e. cyclin B1, cdk1, tubulins, Aurora A, survivin, etc) by a mechanism strictly dependent on PKR expression. In addition, HCV core protein leads to mitotic arrest, inducing nuclear localization of the cyclin B1-cdk1-PKR complex. Ethanol treatment alters the expression of the same mitotic molecules targeted by HCV, but this phenomenon seems to be PKR-independent.

OL-067

Low doses of insulin-like growth factor I (IGF-I) reduce oxidative liver damage and improve glucose and lipid metabolisms in aging rats.

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Background: Age is associated with a reduction of IGF-I levels. Advancing donor age has an adverse influence on graft and survival for patients with chronic liver diseases. Low doses of IGF-I have antioxidant properties on oxidative liver damage in cirrhotic rats[1]. The aim of this work was to study the impact of age in oxidative damage and lipid and glucose metabolisms and the effect of the IGF-I therapy in aging rats.

Methods: Three groups were included: yCO, young controls (17 weeks old); O, untreated old rats (103 w-o); and O+IGF-I, aging rats (103 w-o) treated with IGF-I (1 month, 2.25μg·100g bw^{-1}·day^{-1}). Analytical parameters were determined by routine methods and levels of testosterone, IGF-I and insulin by RIA.

Results: Compared with young controls, old rats showed: a reduction of IGF-I, testosterone and serum total antioxidant status (TAS); increased levels of serum glucose, insulin and HOMA (an index of insulin resistance); elevated cholesterol and triglycerides with a significant reduction of free fatty acids (FFA); and increased levels of malondialdehyde (MDA, a marker of lipid peroxidation) in liver homogenates with a reduction of catalase activity. IGF-I therapy was able to restore all these parameters.

Conclusion: Aging is associated with reduction of IGF-I and testosterone, an increase of oxidative liver damage, insulin resistance and hyperlipidemia. Low doses of IGF-I restore circulating IGF-I and age related-changes improving glucose and lipid metabolism, increasing testosterone levels and serum total antioxidant capability and reducing oxidative liver damage.

[1]Gastroenterology. 1997;113(5):1682-91.

OL-068

Treatment of fatty liver by fatty acid bile acid conjugates (FABACs) in rodents and its mechanism

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Fatty Liver Disease (NAFLD and NASH) is now the most common chronic liver disease in most countries and its prevalence is rising. It may lead to cirrhosis and is presently devoid of therapy. FABACs were shown to prevent NAFLD in animals. This study was designed to test FABAC therapy in established NAFLD and to investigate its mechanism. NAFLD was induced by a High Fat Diet (HFD) in mice and rats. Animals were then treated with Aramchol or Steamchol (25 or 150 mg/kg/day) or placebo while on a full or reduced HFD for 1-3 months. In all 5 studies FABACs significantly ($p<0.03$-0.0002) reduced liver fat (triglycerides). The rate of fat reduction was inversely proportional to the fat concentration in the diet during treatment. The serum 16:1/16:0 fatty acid ratio, a marker of liver Stearoyl CoenzymeA Desaturase1 was reduced by FABACs. Subsequently SCD1 activity was found to be reduced in treated livers as well as in hepatocytes, HEPG2 cells and liver microsomes exposed to FABACs. SCD1 activity was reduced by FABACs more than by Conjugated Linoleic Acid (10trans 12cis). Conclusion: FABACs reduce liver triglycerides by inhibiting SCD1 activity.

OL-069

Low doses of insulin-like growth factor I (IGF-I) induce a liver mitochondrial protection in aging rats

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Background and Aims: In liver transplantation, an old transplanted liver is now an identified cause of primary non-function of the graft and an independent cause of mortality after transplantation. IGF-I

circulating levels decrease with age. We have recently reported that low doses of IGF-I reduce oxidative liver damage with an improvement of antioxidant enzyme activities. Understanding that mitochondria is an important cellular target of IGF-I, the aim was to study the effect of IGF-I therapy on mitochondrial function in aging rats.

Methods: Three experimental groups were included: yCO, young controls (17weeks-old); untreated old rats (103 w-o); and 103 w-old rats treated with IGF-I (1-month, 2.25μg·100g.bw^{-1}·day^{-1}). Liver mitochondrial function was assessed by flow cytometry and caspase-3 by Western-blot.

Results: Compared with yCO, old rats showed an increase of oxidative damage in isolated mitochondria with a mitochondrial dysfunction characterized by: depletion of mitochondrial membrane potential ($p<0.05$) with increased proton leak rates and intramitochondrial free radicals production and a significant reduction of ATPase (all, $p<0.05$). In addition, mitochondrial respiration from untreated aging rats was atractyloside-insensitive, suggesting that adenine nucleotide translocator (ANT) was uncoupled. ANT has been shown to be one of the most sensitive points for opening pore. Accordingly untreated aging rats showed a significant overexpression of the active fragment of caspase-3. IGF-I therapy corrected the parameters of mitochondrial dysfunction and reduced caspase-3 activation.

Conclusion: The hepatoprotective effect of IGF-I in aging is closely related with a mitochondrial protection leading to reduce oxidative damage and apoptosis and to increase ATP production.

OL-070

Alcohol intake aggravates the severity of severe acute pancreatitis

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Objective: To evaluate the effects of alcohol intake before onset of severe acute pancreatitis (SAP) on its outcome.

Methods: Patients with SAP admitted to our hospital from January 2001 to February 2004 were studied. A total of 347 SAP patients were divided into alcohol group (alcohol intake 48 hours before onset of symptoms) and control group (no alcohol intake 48 hours before onset of symptoms). The demographics, laboratories, Ranson's score, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Balthazar's computed tomography (CT) score, complications and mortality were compared.

Results: Comparing with control group (n=270), patients in alcohol group (n=77) had higher male constituent ratio (83.1% vs.41.1%, *P*<0.01), APACHE II score (19.1±5.1*vs.*16.2 ± 6.0, *P*<0.01) and serum triglyceride (5.0±5.0 *vs.* 3.0±3.5, *P*< 0.05) as well as higher incidence rate of acute hepatitis (45.5% *vs.*31.1%, *P*<0.05), acute renal insufficiency (41.6% vs. 26.7%, *P*<0.05), cardiovascular insufficiency (39.0% *vs.* 21.1%, *P*<0.05), shook (35.1% *vs.* 16.7%, *P*<0.01), encephalopathy (35.1% *vs.*15.6, *P*<0.01), infection (26.0% *vs.*15.9%, *P*<0.01) and mortality (39.0% *vs.*5.9%, *P*<0.01).

Conclusions: Alcohol intake before onset of the disease indicates a worse clinical outcome by aggravating the episodes of SAP.

OL-071

A new pattern biomaterials for bioartificial liver

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In this study, to investigate whether induced adult mice hepatocytes by Phenobarbital Sodium（PBS）in vivo and could enhances its biotransformation and smetabolic capability of bilirubin in vitro, and for seeking optimal biomaterials for bioartificial liver. A total twelve adult male mice with 34g mean weight were randomly divided into induced and control group, the mice of induced group were intraperitoneal injected daily with 45mg/kg PBS for 5 days and the control group with normal sodium. The liver tissues was taken from every mouse after 24 hours of last administer, the hepatocytes suspensions were made by divided into each groups, then the jaundice serum and the medium were added into every well of the Culture plate,at the same time has intercalated jaundice serum of no-hepatocytes as control,the culture plate be incubatored for 3 hours, then centrifugation isolated hepatocytes and jaundices serum, the change of bilirubins of serum was determined by Beckmann auto-analyzer. The result of the total and indirect bilirubins reduced by 60% and 71.42%, respectively,in the induced group,and reduced by 34.78% and 54.87%,respectively,in the control group, there were statistic differences in changes of total bilirubin ($P < 0.05$,$t = 2.899$)between the two groups,but showed no statistic differences in changes of indirect bilirubin ($P > 0.05$,$t = 1.571$) between two groups; These results suggest that the adult mice hepatocytes induced by PBS in vivo and can enhances its biotransformation and metabolic activity of bilirubin in vitro，therefore,it may be that the optimal bio material for bioartificial liver.

OL-072

A meta-analysis of endoscopic variceal ligation versus β-Blocker and isosorbide mononitrate for the prevention of variceal rebleeding

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Background: Patients with portal hypertension surviving a variceal bleed are at high risk of rebleeding and mortality. It is still unknown whether combination of β-Blockers and ISMN is superior to EVL as a choice of treatment for preventing variceal rebleeding. The purpose of this study was to perform a meta-analyse of randomized-controlled trials (RCT) comparing EVL with combination of β-Blockers and ISMN for preventing variceal rebleeding.

Methods: A literature search of the Medline, Embase, and Cochrane databases was used to identify randomized controlled trials that evaluated the efficacy and safety of endoscopic variceal ligation in comparison with combination of β-Blockers and ISMN for preventing variceal rebleeding. Meta-analytical techniques were applied to identify differences in outcomes between the two groups.

Results: A total of four studies were identified according to our inclusion criteria, two trials compared the treatment effects of endoscopic variceal ligation (EVL) with combination of Nadolol and ISMN, two trials compared EVL with combination of Propranolol and ISMN. Outcomes for 504 patients were examined. There was no significant difference between the EVL group and β-blockers + ISMN (β+I) group in rate of rebleeding (OR 95%CI, 0.64-1.37; p=0.73), the total mortality (OR 95%CI, 0.50-1.78; p=0.86), the mortality related to esophageal variceal bleeding (OR 95%CI, 0.73-1.70; p=0.61), and the incidence of adverse effects (OR 95%CI, 0.50-1.78; p=0.86).

Conclusions: Combined therapy withβ-blockers and isosorbide mononitrate is as effective and safe as endoscopic variceal ligation for the prevention of recurrent bleeding.

Free Paper Presentation – Molecular & Cellular Biology

OL-073

Liver fluke-induced human cholangiocarcinoma with highly-expressed and modified cytokeratin 19 and albumin may originate from bi-potential stem cell

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Intrahepatic cholangiocarcinoma (ICC) occurs with a higher incidence in Southeast Asia, where *Opisthorchis viverrini* (OV) liver fluke infection is endemic. Chronic inflammation is known to play roles on not only mediating DNA damage but also replicative senescence of cells that triggers progenitor cell activation. To better understand the mechanism of OV-associated cholangiocarcinogenesis, we examined proteins altered in ICC tissues compared with non-tumor tissue from 13 patients and normal liver tissue from 4 cadaveric donors. Proteomic approaches identified highly-expressed cytokeratin19 (CK-19) and albumin in OV-induced ICC tissues compared with non-tumor and normal liver tissues by two-dimensional differential gel electrophoresis analysis and MALDI-TOF mass spectrometer with peptide mass fingerprinting method. Western blot studies confirmed that CK-19 ($r=0.501$, $P<0.01$) and albumin ($r=0.813$, $P<0.01$) expression levels were correlated with the expression level of a progenitor cell marker glypican-3. Double immunofluorescence microscopy studies revealed that the expression of CK-19 was co-localized with albumin and glypican-3 in cytoplasm of the cancer cells of ICC tissue and in the cytoplasm of cholangiocyte with hyperplasia at non-tumor area. In addition, CK-19 and albumin were identified as phosphorylated and glycosylated proteins, respectively. We could demonstrate that OV-induced ICC may possibly originate from bi-potential liver stem cells with mixed phenotypes of albumin expression as hepatocyte and CK-19 expression as cholangiocyte. Moreover, OV infection may induce progenitor cell proliferation via damage of liver tissue in inflammation process and the modification of CK-19 and albumin may associate in tumor progression, leading to ICC development.

OL-074

Genetic polymorphisms and haplotype structures of glucose-regulated protein 78 gene in Chinese Han population

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GRP78 protein is a molecular chaperone that is critical to the folding, maturation and transport of proteins out of the cell. For many diseases which induce endoplasmic reticulum stress concerned with GRP78 gene expression, the polymorphisms and haplotypes should be checked. We amplified GRP78 gene fragments by PCR from genomic DNA isolated from 75 *Han* Chinese. The PCR products were directly applied for DNA sequencing to discover single nucleotide polymorphisms (SNPs) in the promoter region and exons including 5' UTR, CDS and 3'-UTR. Linkage disequilibrium measures and haplotype construction of GRP78 gene were determined using a Bayesian approach implemented with PHASE and the Haploview version 3.2 software. Eleven SNPs and seven possible haplotypes were found in GRP78 gene. Three novel SNPs (-345A/C, +119T/A and +2160T/A) are the first discovery in *Han* Chinese. Among them, SNP +2160T/A causes a missense mutation Asp652Glu. These analyses would provide an important framework for appropriate interpretation of GRP78 gene mutation screening now offered by a number of laboratories for the ancillary diagnoses of virus infection, cancer progress, drug resistance, neurodisease or diabetes. They will assist in the study of GRP78 gene polymorphisms and haplotypes as predictors of complex disease states.

OL-075

Microarray analysis of differentiation of mouse embryonic stem cells into hepatocyte-like cells

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Elucidating the complex molecular regulatory mechanisms underlying hepatic differentiation at early stage contributes both to fully harness embryonic stem cells in liver regeneration, and to understand the differentiation-related liver diseases. Pluripotent embryonic stem cells can be induced into hepatocytes in vitro. mESC-D3, maintained in adherent monolayer culture condition, were induced to differentiate along hepatic lineage with addition of some factors to the medium at different time. The factors included FGF, HGF, OSM and so on. The differentiated cells showed a hepatocyte-like morphology, expressed hepatic marker genes and have also produced and stored glycogen by phase contrast and transmission electron microscopy, reverse transcription-polymerase chain reaction, immunocytochemistry, and periodic acid-Shiff staining respectively. Stem cell differentiation-related microarray was used to analyze the differential gene expression profiling during hepatic differentiation of mESC-D3. Quantitative PCR was performed to verify the microarray data.. Microarray analysis presented 48 genes expressed differentially (2 fold), including 20 genes up-regulated and 28 genes down-regulated. Further bioinformatics analysis showed the majority of these genes were extracellular matrix, intercellular junction and FGF, BMP, Notch and Wnt signaling pathways molecules, which suggests these alterations may be closely associated with the hepatic differentiation of embryonic stem cells at early stage.

OL-076

Retinol binding protein-4 expression is up regulated in rats with fatty liver during quiescent and regenerative state and improves with insulin sensitizing agents

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Background: Serum RBP-4 levels are increased very early in insulin resistance states.

Aim: 1) Determine whether there is a relationship between NAFLD and RBP-4 levels in quiescent and in regenerating rat fatty liver 2) determine the effect of insulin sensitizing agents (ISA) on RBP4 expression and on other metabolic parameters.

Methods: Forty-eight SD rats were treated with fructose enriched diet (FED), or FED with Metformin (2 mg/Kg/d), FED with Rosiglitazone (3 mg/Kg/d), or the combination of both drugs for a total of 5 wks. 30% PHX was performed at WK 5. RBP-4 expression, lipids, antioxidants, and hepatic fat content were measured before 24, and 48 hours after PHX.

Results: Hepatic and serum RBP-4 were significantly higher in rats with fatty liver than control rats (+22%, +52%, $p<0.01$). RBP-4 was independent factor associated with NAFLD ($r=0.4$, $P<0.001$) and the extent of hepatic RBP-4 expression increases significantly 24 and 48 hours after PHX. (+90%, $p< 0.001$, +47%, $p<0.001$) respectively. The combination of metformin and rosiglitazone decreased hepatic and serum RBP-4 expression at 48 hours after PHX by -55% and -45% respectively. Serum RBP-4 correlate positively with MDA levels ($r=0.4$, $P<0.01$), TG levels ($r=0.23$, $p<0.01$), and markers of liver regeneration

Conclusion: Hepatic and serum RBP-4 levels are highly associated with fatty liver, are up regulated during liver regeneration and improve after treatment with insulin sensitizing agents.

OL-077

SiRNAs targeted against cyclin E1, cyclin E2 and E2F1 down regulate the growth of hepatocellular carcinoma showing their potential use in the prevention of this type of tumor.

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The over-expression of CyE1 in about 70% of HCC patients, correlates with a poor prognosis and life expectancy is short. These observations prompted us to deplete CyE1 together with CyE2 and E2F1, cooperating to promote cell growth, and to test their anti-proliferative effect in HCC cells. siRNAs were delivered via-liposomes to cultured HepG2, JHH6 and HUH7 cells, undertaken as 3 grades of HCC differentiation. The effects of siRNAs on protein and mRNA levels, cell proliferation, apoptosis and citotoxicity were evaluated. Every specific siRNA tested was able to strongly reduce mRNA and protein

levels of the respective target. In parallel, a reduction in the number of cells was observed. Three days after transfection, a pronounced decrease of S-phase cells and a moderate accumulation of cells in G1 were observed. Depletion of CyE2 caused an increase of CyE1 protein, without affecting mRNA levels. This in part was due to a reduced CyE1 protein degradation, as tested by a reduction in CyE1-phosphorylation (Thr 395). In HepG2, but not in HUH7 and JHH6, depletion of CyE1, CyE2 and E2F1 induced apoptosis. Finally, no cytotoxic effects and no siRNA-triggered interferon response were observed. The presented results indicate, for the first time, that the depletion of CyE1, CyE2 and E2F1 is effective in preventing HCC cell expansion and showing the therapeutic potential of our siRNAs in the prevention of HCC development. Moreover the presented research can contribute to find novel therapeutical approach for HCC and to understand the complex mechanisms regulating cell proliferation in tumor cells.

OL-078

Capillarisin attenuates glycochenodeoxycholic acid-induced oxidative stress in rat primary hepatocytes

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The accumulation of hydrophobic bile acids plays a role in the induction of apoptosis and necrosis of hepatocytes during cholestasis. Glycochenodeoxycholate acid (GCDC) trigger a rapid oxidative stress response as an event of glutathione (GSH) depletion and nuclear factor kappa B (NF-κB) activation. We therefore investigated whether the antioxidant bioactivity of capillarisin (Cap) prevents GCDC-induced hepatocyte damage.

Isolated rat hepatocytes were co-incubated with GCDC 100 μM and Cap 0.5 mg/ml for 4 hours. GSH depletion and thiobarbituric acid reactive substances (TBARS, measure of lipid peroxidation) increased after GCDC exposure but were markedly suppressed by Cap treatment. Flow cytometry analysis shown that Cap attenuated GCDC-induced hepatocytes damage was accompanied by a decreased generation of reactive oxygen species (ROS) and a reduction of mitochondria membrane potential. In addition, Cap has shown to inhibit GCDC-mediated NF-κB activation by using electrophoretic mobility shift assays (EMSA). In contrast to GCDC, Cap not only significantly decreased cytochrome c release and caspase-3 enzyme activity but also suppressed heme oxygenase-1 protein and mRNA expression in hepatocytes.

These data indicated that Cap may prevent hepatocytes from GCDC-induced apoptosis, the down-regulation of heme oxygenase-1 expression and a decrease in ROS generation are involved.

OL-079

Up-regulation of Bak by ZBP-89 in hepatocellular carcinoma (HCC)

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Bak is a pro-apoptotic Bcl-2 family member and zinc finger binding protein-89 (ZBP-89) is a Kruppel-type transcription factor. ZBP-89 is known to inhibit tumor growth but its role is largely unknown. The purpose of this study was to investigate how ZBP-89 affects cell death of HCC cells (SK-Hep-1). The result showed that ZBP-89 significantly reduced the proliferation of SK-Hep-1 by promoting apoptosis. The level of Bak was obviously enhanced in the cells infected with AdZBP-89 and the blockade of ZBP-89 with its siRNA lead to a reduction of Bak level. Our results suggest Bak is positively regulated by ZBP-89. Luciferase reporter assay and electrophoretic Mobility Shift Assay were done to identify the possible interaction between ZBP-89 and the Bak promoter. It showed that ZBP-89 may act on Bak via a response element located between -1529 and -1745bp upstream of the AUG Bak start codon. In conclusion, ZBP-89 inhibits the proliferation of HCC cells via induction of apoptosis. The apoptosis induced may be attributed to the binding of ZBP-89 to the promoter of Bak of which leads to an enhancement of Bak production. (This work was supported by the Research Grants Council of Hong Kong SAR: CUHK4551/05M).

OL-080

Oxysterol binding protein L7 as a potential marker for diagnosis of cholangiocarcinoma

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Oxysterol binding protein (OSBP) is the cytosolic protein with high affinity receptor for oxysterols, which are oxygenated derivatives of cholesterols derived from enzymatic reaction or free radical oxidation and have significant physiological and pathological effects. Overexpression of some OSBP isoforms was demonstrated in various solid cancers. We previously reported the differential expression of OSBPL8 isoform in CCA tissue of hamster compared with normal gall bladder epithelia, screened by fluorescence differential display suggesting that this gene product may serve as a diagnostic tool for CCA. This study aimed to identify the expression pattern of OSBP isoform(s) in human CCA tissue and CCA cell lines by real time RT-PCR and immunohistochemistry. The results showed that OSBPL7 and OSBP2 expressions were up-regulated in CCA tissue (n=8) to 25 and 4 folds respectively, whereas OSBP1 and OSBPL8 showed down-regulation to 3 and 1.6 folds, respectively when compared with cadaveric liver donors (n=8). The expression of OSBP isoforms could also be detected in 4 CCA cell lines but at different levels. Investigation in CCA cell lines, KKU100 revealed the lowest expression of all detected isoforms. M139, M214, M213 showed higher expression of OSBPL7 when compared to KKU100. Localization study using immunohistochemical technique confirmed that OSBPL7 protein significantly increased in 5 of 8 cases (62.5%) whereas normal biliary epithelia, hepatocytes and inflammatory cells showed negative staining. We concluded that OSBPL7 may be a potential candidate protein served as a diagnostic marker for CCA.

OL-081

Functional analysis of three different isoforms of osteopontin in human hepatocellular carcinoma

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Osteopontin (OPN), a member of SIBLING family, encodes three splice variants (OPN-1, OPN-2, OPN-3). In our previous study, OPN was identified as one of the key genes involved in the invasion and metastasis of hepatocellular carcinoma (HCC). However, the roles of the three different isoforms of OPN (OPN-1, -2, and -3) in HCC have not been shown. In this study, we investigated the expression levels of these 3 isoforms of OPN in 100 cases of human HCCs with real-time quantitative PCR, and found that the expression levels of OPN-1 and OPN-2 are 10 folds of that of OPN-1（$p<0.01$). To clarify the role of these isoforms and to better understand their function in HCC, we ectopically expressed each OPN isoform in HepG2 cell line. In HepG2 cells, expression of exogenous of OPN-1 and OPN-2 resulted in an increased cell adhesion ($p<0.05$) compared with OPN-3. Overexpression of OPN-1 and OPN-2 activated the signal transducers and activators of transcription 3(STAT3) and extracellular signal-regulated kinase(ERK) signaling pathways, however OPN-3 could not. These results underlined the importance of the multiple isoforms and different consequences on gene expression.

Free Paper Presentation – Transplantation

OL-082

Comparison of four model for end-stage liver disease-based prognostic systems for cirrhosis

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Background/Aim: Serum sodium (Na) has been suggested to incorporate into the model for end-stage liver disease (MELD) to enhance its prognostic ability for cirrhotic patients. Three Na-containing models, MELD-Na, integrated MELD (iMELD) and MESO (MELD to sodium ratio), were independently proposed for this purpose. This study investigated the accuracy of the four MELD-based models for outcome prediction.

Methods: The c-statistic equivalent to the area under receiver operating characteristic curve (AUC) to predict 3- and 6-month mortality was compared in 825 cirrhotic patients.

Results: The MELD score tended to be lower with increasing Na level. At 3 months, the iMELD had the highest AUC (0.807), followed by the MEND-Na (0.801), MESO (0.784) and MELD (0.773); the difference between MESO and MELD was statistically significant (p=0.013). At 6 months, the iMELD still had the highest AUC (0.797), followed by the MEND-Na (0.778), MESO (0.747) and MELD (0.735); all comparisons showed significant differences between each other (p all < 0.01), with the exception between iMELD and MELD-Na (p=0.18). Using the most discriminative cutoffs, the specificity and negative predictive value were 71-85% and 89-97%, respectively, at 3 and 6 months for the four models. Patients with spontaneous bacterial peritonitis (SBP) consistently had significantly higher MELD-derived scores in all four models in comparison with patients without SBP (p all < 0.01). Patients with hepatic encephalopathy also had higher scores in all four models, although the statistical significance was established only for the iMELD (41.0±11.5 vs 37.6±9.1, p=0.037).

Conclusion: Incorporation of Na into the MELD may enhance its prognostic accuracy. Both iMELD and MELD-Na are better prognostic models for outcome prediction in cirrhosis. Patients with SBP have a higher MELD-derived score.

OL-083

The role of entecavir in preventing hepatitis B recurrence after liver transplantation

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Background: Liver transplantation is the only effective treatment for end-stage liver disease. Though hepatitis B recurrence after liver transplantation has reduced to 0%~10% since the application of the combination therapy with hepatitis B immunoglobulin (HBIG) and lamivudine, the viral mutation resistance of lamivudine is still an obstacle for the outcome of liver transplantation. We aim to evaluate the role of entecavir, a deoxyguanine nucleoside eanalogue, in preventing hepatitis B recurrence after liver transplantation.

Methods: Among 247 patients with hepatitis B related disease who underwent liver transplantation in our center between March 2006 and December 2007, 14 patients received entecavir (0.5mg orally, daily) together with long-term low-dose of HBIG to prevent hepatitis B recurrence after transplantation. Three patients who died within one week after surgery were excluded from the 14 patients. Of these eleven patients, eight (72.7%) were HBV-DNA positive and three (27.3%) were HBeAg positive before transplantation.

Results: The median follow-up time was 12.6 months. No patients were detected hepatitis B reinfection. The hepatitis B surface antigen of all patients became negative within one week. No patients had side-effects related to entecavir. One patient died of infective endocarditis five months after transplantation. All other ten patients survive and are followed up currently.

Conclusions: The study shows that entecavir is effective and safe to prevent hepatitis B recurrence after liver transplantation, but the long-term effect is still on the investigation and large-sample research should be carried out in the future.

OL-084

Impact of combined of mycophenolate mofetil and low dose calcineurin inhibitors therapy on T cell function in vivo and in vitro

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Aim: We investigated the impact of combined mycophenolate mofetil (MMF) and low dose CNI therapy on T cell function in vivo and in vitro.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from 32 liver transplant recipients at baseline and 12 months after conversion to MMF/low dose CNI regimen. T cell frequencies, phenotype and expression of activation markers were analyzed by flow cytometry. In in vitro studies, freshly isolated PBMC from healthy volunteers were treated with mycophenolic acid (MPA), cyclosporine A (CsA), tacrolimus (Tac) and combined CsA/MPA or Tac/MPA to assess the effect on T cell proliferation, expression of activation markers and of nuclear factor of activated T cell (NFAT)-regulated cytokines.

Results: Combined MMF and low dose CNI therapy in vivo leads to a reduction of the percentages of CD8+ and CD8+CD56+ T cells, to inhibition of T cell activation and to an increase of circulating FOXP3+CD25+CD4+ regulatory T cells (Treg) as compared to CNI monotherapy. MPA stops the cell cycle of activated T cells at G0/G1 phase in vitro; whereas MPA and CNI each individually inhibit the expression of T cell activation markers, and this effect is enhanced by combined exposure to MPA and CNI. Low doses of CNI strongly inhibit interleukin (IL)-2, IL-4, IL-10 and interferon (IFN)-γ production by T cells in vitro.

Conclusions: Immune monitoring may facilitate optimization of the immunosuppressive regimen with minimization of drug related adverse effects without increasing the risk of cellular rejection.

OL-085

Liver transplantation outcomes: The relation of post transplantation MELD and rejection.

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Background: Since 2001 the new rules propose to replace the Child's classification, as the basis for determining urgency for liver transplantation, with the Mayo End-stage Liver Disease (MELD) score. In this model only bilirubin, INR and creatinine could predict the patients' outcome. In this study we want to know if this model could be a predictor of early liver transplantation rejection**.**

Patient: In this study we selected all the patients who underwent liver transplantation in the only organ transplantation center in Shiraz, Iran in the previous year (83-84). The reason for this selection was to avoid any surgical method complications since we have obtained enough experience during these years. In all patients rejection was based on elevation of liver enzymes or other clinical findings such as fever. Any surgical complications or expired patients were excluded.

Methods: The mathematical measure to determine the validity of the model was the concordance C-statistic (equivalent to the area under receiver operating characteristic curve (ROC)).This statistic may range from 0 to 1 with 1 corresponding to perfect discrimination and 0.5 to what is expected by chance alone. The closer this statistic to 1 the more it can predict the event. Finally the MELD scores in each day were analyzed separately and the closest C-statistic to1 assumed the day which could predict rejection.

Results: Among 61 patients included in this study the mean of age were 36.67±12.23 (range 12-57) years. In our study group 37 were males and 24 were females. The highest C-statistic was belonging to the 16th day which was 0.96 and the next one was belonging to the 20th day which was 0.90.

Conclusion: In patients who underwent transplantation the MELD score in the 16th day of post transplantation can predict patients' rejection accurately.

OL-086

The value of plasmapheresis direct before liver transplantation for the patients with acute on chronic severe hepatitis: A report of 82 cases

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Objective: To observe and investigate the efficacy of plasmapheresis (PP) direct before liver transplantation on the operation and prognosis in acute on chronic severe hepatitis patients.

Methods: Consecutive 82 patients with acute on chronic severe hepatitis underwent cadaveric(72 cases) or right lobe living donor(10 cases) liver transplantation from June 2004 to December 2007 were analyzed retrospectively. The patients were allocated to two groups based on PP or not. The PP group consisted of 56 patients, these patients were treated with PP 2-3 hours before operation. The control group consisted of 26 patients. No differences in sex distinction, mean age, disease distribution and scores of model for end-stag disease (MELD) were found between the PP group and the control group. The operating time, volume of blood loss, volume of blood infusion, the process of recovery, the postoperative complications and the survival rate were observed and compared.

Result: Among 82 cases, a overall one-month survival rate was 84.16 % (69/82), a overall one-year survival rate was 76.92% (50/65). The volume of blood loss, volume of blood infusion and the operating time were remarkably lower in the PP group than in the control group (P<0.05). Consciousness, digestive system recovered more early in the PP group than in the control group. There were no significant differences of complications and the proximate as well as long-term survival between the two groups after liver transplantation.

Conclusions: PP not only can not reduce the perioperative complications but also can not improve the survival rate among the acute on chronic severe hepatitis patients who underwent liver transplantation as well. However, the application of fresh plasma exchange directs before liver transplantation on patients with acute on chronic severe hepatitis is facilitated to the operative procedure and the postoperative recovery course.

OL-087

Prophylaxis effect of recombinant adeno-associated virus type 1 vector containing HBsAb Fab fragment combined with HBIG on HBV reinfection after liver transplantation in tupaia

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We have constructed rAAV1 containing an anti-HBs-Fab genes, which derived from phage antibody library, rAAV1–Fab, in our preliminary experiment. This study was designed to examine the efficacy of the rAAV1–Fab on HBV reinfection after liver transplantation in tupaia and evaluated the synergistic effect of HBIG. Adeno-associated virus type 1 encoding the Fab gene was introduced intra-portally into liver grafts and clamped for 30min during cold preservation. Those tupaias were injected intra-peritoneally with purified HBV immediately after recanalization of portal vein. Administration of rAAV1–Fab resulted in reduction in HBsAg level followed by recurrence to initial levels within few days. But when we combined rAAV1–Fab with HBIG, we found that HBIG quickly decreased the viral load and prolonged incubation period of HBV which was important for anti-HBs-Fab genes express in tupias. For the complementary action of the two administrations, it was more effective in prevention of HBV liver recurrence and reduction of viral load. Thus, rAAV1–Fab combined with HBIG may be potential candidates for preventive therapy

OL-088

Liver transplantation in elderly recipients: a report of the liver transplantation database in China

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Background: There has been debate about whether the elderly recipients have as the same result as the younger recipients after LT. In recent years, although the development of LT is rapid in China, little research about LT in elderly recipients has been done.

Methods: Clinical data were retrospectively analyzed in 250 patients receiving LT. Elderly recipients aged 60 years and over were 36 cases (older group) and the younger recipients less than 60 years were 214 cases (control group). The differences between both groups in preoperative, intraoperative and postoperative variables, including main clinical and laboratory features, were analyzed.

Results: Pulmonary infection was the cause of death in 5 patients in older group with 41.7% higher than that in control group ($P<0.05$). The patient survival rates at 1, 2, and 3 years in older group were 77.8%, 71.8%, and 61.6%, and in control group 85.5%, 72.2% and 65.5% ($P>0.05$), but the patients with liver carcinoma exceeding Milan criterion had a significantly lower survival rate in older group than in controlled group ($P<0.05$).

Conclusions: Overall survival rate in older group is similar to that in control group and LT is not of contraindication for elderly recipients. The elderly recipients with liver carcinoma exceeding Milan criterion have a significantly lower survival rate than the younger recipients, so LT is not suitable for them. Infection rate in older group after LT is higher and pulmonary infection is the most main cause of death.

OL-089

Endoscopic treatment with multiple stents for post-liver transplantation nonanastomotic biliary strictures

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Introduction: Biliary nonanastomotic strictures (NAS) occur in 2-20% of post-orthotopic liver transplantation (OLT) patients and are associated with increased morbidity and mortality. We evaluated endoscopic retrograde cholangiopancreatography (ERCP) with multiple stents as a treatment modality for NAS.

Methods: Fourteen patients with post-OLT NAS were referred for ERCP with dilation and stenting. Stents were only changed when signs or symptoms of obstruction were noted. The primary outcome was NAS resolution, defined by absence of significant cholangiographic bile flow impairment, clinical symptoms of biliary obstruction, and elevated serum bilirubin, or treatment failure, defined by need for re-OLT or death due to biliary causes.

Results: Median time from OLT to NAS was 9.5 months. Nine patients completed treatment, of whom 8 (89%) achieved resolution and 1 (11%) required re-OLT. Of the 5 remaining, 1 died of recurrent hepatoma and 4 still have stents in place with normal bilirubin. The 8 resolved patients had 8.8 (±4.5) (mean ±SD) total stents, 1.9 (±0.7) stents per ERCP, 4.8 (±2.3) ERCPs, and 20.6 (±28.8) months from OLT to NAS diagnosis. Complications included a retained basket that was removed by interventional radiology and 1 case of post-ERCP fever out of 68 total ERCPs. During 8 month median follow-up, none of the 8 successfully treated patients had NAS recurrence.

Conclusion: Endoscopic treatment of NAS with multiple stents appears to be effective, safe, and infrequently associated with NAS recurrence, and it may reduce need for re-OLT.

OL-090

Monocyte-derived Hepatocyte-like Cells increases the Survival in a Model of Acute Liver Failure

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Recent evidence suggests that cells of monocytic origin can transdifferentiate into hepatocyte-like cells. Since extended liver resection or split liver transplantation is associated with postoperative liver insufficiency, we investigated the efficacy of hepatocyte-like cell implantation in a model of surgically induced liver failure. In a model of acute liver failure, cells of different origin were injected into the spleen of Wistar rats before surgery. Five days after surgery, postoperative weight, signs of encephalopathy, blood parameter and animal survival were improved in animals that had received hepatocyte-like cell transplantation.

Free Paper Presentation – HBV

OL-091

Envelop antigen status is associated with HBV-specific T-cell response and liver damage in patients with chronic hepatitis B

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Aim: To study the quantity, phenotypic characters and function of HBV-specific T-cell in chronic hepatitis B (CHB) patients with different HBeAg status, and to investigate the association between HBeAg and liver damage.

Methods: 103 CHB patients were divided into two groups according to the HBeAg status, and the serum HBV markers, liver damage were analyzed. The number and Foxp3 expression of regulatory T cells (Treg), HBV-specific T-cell frequency and the expression of PD-1, CTLA-4 were measured. The HBV antigens specific T-cell responses were also observed.

Results: The serum ALT, AST levels, the frequency and Foxp3 expression of Treg were similar between HBeAg- and HBeAg+ patients, while HBV DNA levels were higher in HBeAg+ patients (P <0.05). The liver necroinflammation was more severe in HBeAg- patients (P =0.056), but the median percentage of liver cirrhosis was much higher in HBeAg+ patients (P <0.05). The difference of HBV-specific T-cell frequency was not significant between HBeAg+ and HBeAg- patients, while the expression levels of PD-1 and CTLA-4 on HBV-specific CD8 T cells were significantly higher in HBeAg+ patients (P both <0.05). Combined using of anti-PD-L1 and anti-CTLA-4 could significantly increased the cellular proliferation in either HBeAg+ or HBeAg- patients, but only markedly enhanced the IFN-γ production in HBeAg+ patients.

Conclusion: The persistency of HBeAg could induce higher expression of PD-1 and CTLA-4 on the HBV-specific T cells, which may associate with the low ability of HBV-specific T-cell responses, high HBV DNA levels and high percentage of liver cirrhosis in HBeAg+ CHB patients.

OL-092

Characterization of circulating CD4+CD25+ regulatory T cells in different stages of HBV infected patients

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Aims: To identify the frequency and suppressive function of circulating $CD4^+CD25^+$ regulatory T cells (Treg) in different stages of HBV infected patients, and to investigate the role of Treg in the progress of chronic HBV infection.

Methods: A total of 137 subjects including 79 chronic hepatitis B (CHB) patients, 26 asymptomatic HBV carriers (ASC), 12 acute hepatitis B (AHB) patients and 20 healthy controls were enrolled. The frequency, phenotypic characters and cytokines production of circulating $CD4^+CD25^+$ Treg were analyzed. The suppressive ability and mechanism of Treg upon antiviral effector T-cell were also observed in vitro.

Results: The frequency of $CD4^+CD25^{high}$ (the mean fluorescence intensity, MFI>50) T cells in CHB patients was comparable to that of ASC, while it was significantly reduced in AHB patients and healthy controls. In each group with different HBV infection stage, $CD4^+CD25^+$ Treg produced interleukin (IL)-10 but little or no IFN-γ under anti-CD3 stimulation. And, in CHB patients, the frequency of $CD4^+CD25^+$ Treg positively correlate with serum viral load, and the Treg were capable of suppressing proliferation and IFN-γ production of autologous PBMC mediated by HBV antigens stimulation in vitro. Combined administration of anit-PD-L1 and anti-CTLA-4 mAb, but not anti-IL-10 and/or anti-TGF-β mAb, significantly restored cellular proliferation whereas partially increased IFN-γ production of PBMC coclutured with Treg.

Conclusion: The frequency of circulating $CD4^+CD25^+$ Treg was significant increased in CHB patients, which was positively associated with HBV DNA load. Treg appear to play an important role in viral persistence by modulating virus-specific immune responses in CHB patients.

OL-093

The hepatitis B small surface antigen induces extracellular secretion of cyclophilin A: experimental studies and clinical implications

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Cyclophilin A (CyPA), the major target for the potent immunosuppressive drug cyclosporin A, was found decreased significantly both in HBsAg positive transgenic mouse livers and in a subpopulation of liver samples from HBV infected patients. To analyze the molecular mechanisms of interactions between HBsAg and CyPA, hepatitis B small surface antigen was transiently transfected into Huh7 cells. Increased CyPA secretion was induced by the expression of hepatitis B small surface antigen and intracellular CyPA showed direct binding to HBsAg. However, once secreted, extracellular CyPA lost this binding competency. Increased CyPA secretion was confirmed by higher CyPA levels detected in the sera of HBV infected individuals and HBsAg positive transgenic mice, compared to the controls. CyPA belongs to the immunophilin family, is a ubiquitously distributed and abundantly expressed intracellular protein. Recently it was found to be secreted from cells in response to oxidative stress and inflammatory stimulations, promotes inflammation, vascular smooth muscle cell (VSMC) growth, and endothelial cell apoptosis. This is the first report to show that expression of hepatitis B small surface antigen is another factor leading to CyPA secretion. In addition, CyPA was reported as a new type of chemokine. By hydrodynamic injection of HBV DNA in mice, the expression of HBsAg in hepatocytes was found associated with filtration of inflammatory cells in liver sections. These findings are important for understanding the pathogenesis caused by the small S protein of HBV, and may lead to future development of effective measures for converting HBsAg positive to negative in patients.

OL-094

Chemical genetic screening of small-molecule inhibitors of hepatitis B virus replication

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Introduction: Chemical genetics is the study of gene-product function in a cellular or organismal context using exogenous ligands. In this approach, small molecules that bind directly to proteins are used to alter protein function, enabling a kinetic analysis of the in vivo consequences of these changes. The objective of this study is to establish a high-throughput screening (HTS) platform to isolate and identify novel small molecules which can inhibit the hepatitis B virus (HBV) replication based on the concept of chemical genetics.

Methods: HepAD38 cell line was used as an in vitro HBV infection model. Using HBeAg as the selection marker, the 384 well anti-HBeAg cytoblot was performed to screen the small molecule inhibitors of HBV replication from a chemical library. The parallel 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay was applied to determined the cytotoxicities of small molecules.

Results: The expression levels of HBcAg, HBeAg and HBsAg in HepAD38 cells were all measured by cytoblot assays, and only the OD450 values of anti-HBeAg cytoblot were over 4 times difference before and after the inducement of HBV replication, which was suitable for high throughout screening. Using the 384 well anti-HBeAg cytoblot as the HTS platform, 960 small molecules were screened and 7 molecules exhibited potent antiviral activities (over 30% inhibiton of HBeAg production). Among these molecules, 5 molecules had no significant cytotoxicities as revealed by MTT assay.

Conclusions: The 384 well anti-HBeAg cytoblot can be used as a HTS platform to isolate and identify small molecule inhibitors of HBV replication.

OL-095

Management of chronic hepatitis B (CHB) antiviral resistance – The Asia experience

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Background/Aims: More antiviral agents are now available for the treatment of CHB. However, emergence of resistance to antivirals remains a challenge, given long-term treatment is required for most

patients. Studies have shown clinical benefits are reduced or lost once resistance develops. Furthermore, cross-resistance limits future treatment options.

Methods: In most of the Asian countries, physicians have limited access to resistance testing. Using a face-to- face interview questionnaire, this survey aims to identify how physicians diagnose and manage antiviral resistance in the absence of resistance tests. The study randomly selects 565 CHB-treating physicians from China, South Korea, Taiwan, and Thailand where prevalence of HBV infection is intermediate to high, making it a substantial burden on the healthcare systems both clinically and financially.

Discussion: The survey contains two components: (1) the clinical component aims to understand current clinical practice of diagnosing antiviral resistance, particularly the clinical parameters used to identify "suspected" antiviral resistance in the absence of specific resistance tests, and how resistance is managed with regards to modification of antiviral regimen; and (2) the cost component aims to estimate financial impacts of managing "suspected" resistance in both outpatient and inpatient settings. Expected in May 2008, findings from this survey would provide information on physician's approach to managing development of resistance to antiviral therapy when specific resistance tests are not available and form a basis for enhancing CHB treatment guidelines on managing antiviral resistance in Asia Pacific.

OL-096

Reduced expression of toll-like receptor 2 on peripheral monocytes in chronic HBeAg positive patients

Yaping Han, Jun Li, Yufeng Wan

Objective: In order to observe the relationship between the TLR2 and HBeAg、HBV-DNA on the base of the research in the CHB,we analyzed the expression level of TLR2 on PBMC in chronic hepatitis B virus infection. Meanwhile, we also analyzed the TLR2 expression level on PBMC after excited by ligand of TLR2 (Pam3CSK4).

Methods: 49 samples of CHB patients including 37 HBeAg positive , 7 HBeAg negative and HBV-DNA negtive, 5 HBeAg negative and HBV-DNA positive and 16 healthy volunteers were reserched. We collected peripheral blood from the above samples and cultured the cells with Pam3CSK4 in 3 hours .We compared TLR2 expression on CD14+ cells before exited to the excited cells by FACS Calibur. We also anlyzed the relationship of the expression level of TLR2, HBeAg and HBV DNA.

Results:We found that the TLR2 expression level on CD14+ PBMC was significantly decreased in HBeAg positive samples (47.57±21.40 %) compared to both healthy volunteers (76.51±7.46%) and HBeAg negative samples（74.94±11.69 %）, but there was no difference between HBeAg-negative patients and healthy volunteers. We also demonstrated that expression level of TLR2 on PBMC was significantly increased after TLR2 ligand excited in HBeAg positive patients, and could achieve the level of controls before excited.

Conclusions: In the presence of HBeAg, HBV down-regulates the expression of TLR2 on PBMC. Pam3CSK4 can elevate the TLR2 expression in HBeAg-positive patients. Cordingly, the TLR2 expression level in CHB patients may be can conduce to elucidate the effect of immunity factor in the development of hepatitis.

OL-097

Clevudine is highly effective in HBV DNA suppression in HBe antigen-negative chronic hepatitis B

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Background and Aims: Clevudine is a pyrimidine analog with potent and sustained antiviral activity in chronic hepatitis B (CHB). This study aimed to evaluate the antiviral activity of clevudine for 48 weeks.

Patients and Methods: A total of 87 patients with CHB received 30mg clevudine for 48 weeks and were assessed for serum aminotransferase, HBV DNA levels, and HBeAg from baseline to week 12, 24, 36, and 48, respectively.

Results: The sex ratio was 61:26 (male:female) and the mean age was 45.4□12.3 (17-72). Mean serum ALT and median baseline HBV DNA were 117.8□127.6 U/L and 4.98□4.47 X10^7copies/mL respectively. HBeAg was positive in 59 patients and negative in 28 patients. Assessment at 48 week showed normalization of serum ALT in 81 patients (93.1%), undetectable serum HBV DNA (<2000 copies/mL) in 57 patients (65.5%) and HBeAg loss in 12 patients (20.3%) from 59 HBeAg positive patients. Undetectable serum HBV DNA levels were significantly frequent in HBeAg negative patients (25/28, 89.3%) compared with HBeAg positive patient (32/59, 54.2%, p=0.003). The undetectable serum HBV DNA level was achieved at 68.9% (42/61) in non-cirrhosis patients and 68.9% (42/61) in cirrhosis patients (p=0.631). Baseline serum ALT and HBV DNA levels were not related with the undetectable serum HBV DNA levels (p=0.839, p=0.868, and p=0.121, respectively). Loss of HBeAg was not affected by age (p=0.198), clinical cirrhosis (p=0.623), baseline ALT (p=0.460), and baseline HBV DNA level (p=0.959).

Conclusion: Clevudine showed a profound efficacy in HBV DNA suppression especially in HBeAg negative CHB patients at 48 weeks therapy.

OL-098

Detection of angtigen-specific cytotoxic lymphocytes and their association with clinical status in patients with hepatitis B

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Background/Aims: To investigate the difference of host immune response specific to hepatitis B virus (HBV) infections between acute self-limited and chronic persistent hepatitis by quantitative analysis of HLA-A0201 restricted antigen-specific cytotoxic T lymphocyte cells (CTL).

Methods: The frequency and function of HBV-specific CTL cells in the peripheral blood mononuclear cells (PBMCs) from 8 patients infected with HBV were quantified by enzyme-linked immunospot (ELISPOT) assays using three HLA-A0201 restricted HBV epitopes, 3 with acute hepatitis B and 5 with chronic hepatitis B.

Results: High frequencies of circulating antigen-specific CTL cells were detected in most individuals with acute HBV infection. Antigen-specific CTL cells were not detected in the PBMC from individuals with chronic HBV infection.

Conclusions: HBV antigen-specific CTL cells may play a crucial role in complete clearance of HBV from patients with acute HBV hepatitis. The proposed function of HBV antigen-specific CTL may provide an important approach as to the treatment for the persistent HBV infection.

OL-099

Outcome and immune reconstitution of HBV-specific immunity in patients with reactivation of occult HBV infection after alemtuzumab-containing chemotherapy regimen

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Background: Whether preemptive anti-hepatitis B virus (HBV) therapy should be considered in all hepatitis B surface antigen (HBsAg)-negative patients with occult HBV infection receiving alemtuzumab-containing chemotherapy is uncertain.

Aim: To determine the outcome and effect of HBV-specific immunity of alemtuzumab-containing regimen in occult HBV-infected patients.

Methods: Twenty-one consecutive occult HBV-infected patients treated with alemtuzumab-containing chemotherapy regimen were studied. T cell reactivity to HBV-antigens and peptides were quantified by ELISpot and T cell subset by flow cytometry.

Results: Six of the 21 patients (28.6%) developed HBsAg seroreversion. The median (range) time to development of HBsAg seroreversion after the end of chemotherapy was 1.8 (0.2-2.3) months. Direct sequencing showed that the occult HBV infection in all 6 patients (100%) was reactivated. These 6 patients developed severe HBV-related hepatitis. At the end of follow-up, 4 of these 6 patients (66.7%) had become negative for HBsAg. Recovery of CD4+ T cell count and CD4+ T cell reactivity against hepatitis B core (HBc) antigen occurred at 9 months after en of chemotherapy. Loss of HBsAg occurred after recovery of CD4+ T cell count and increased CD4+ T cell reactivity against HBcAg 9 months after end of chemotherapy.

Conclusion: Alemtuzumab-containing chemotherapy regimen is associated with a high risk of reactivation of occult HBV infection. Suppression of HBV immunity by alemtuzumab-containing

chemotherapy regimen would persist until 9 months after end of chemotherapy. In occult HBV-infected patients receiving alemtuzumab-containing chemotherapy regimen, preemptive anti-HBV therapy should be continued until 9 months after end of chemotherapy, when recovery of HBV immunity has occurred.

OL-100

Retinoic acid-inducible gene I expression and function of monocyte-derived Dendritic cells in patients with hepatitis B virus infection

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Background: The retinoic acid-inducible gene I (RIG-I)can recognise viral RNA and result in IFN-β induction. Dendritic Cells function as the most important part of the native immunity against virus.This study aims to investigate the function of RIG-I in Monocyte-derived Dendritic Cells (MoDC) at various stages of HBV infection, to explore the role of RIG-I in the disease progression after HBV infection.

Methods: Peripheral blood was collected from 28 hepatitis B virus-infected persons,including 21 chronic hepatitis B (CHB),7 acute hepatitis B (AHB),and 18 healthy controls.Purified Monocytes were isolated by CD14 Microbeads.Cells were cultivated with GM-CSF and IL-4 for 7 days,then infected with vesicular stomatitis virus (VSV) to stimulate RIG-I.The expression of RIG-I,IFN-promoter stimulating factor 1 (IPS-1) and IFN-β at mRNA level after infection with VSV at 16h and 24h were analyzed by Real-time PCR.

Results: The expression level of RIG-I in MoDC of CHB were significantly lower than in AHB and healthy ($P < 0.05$); the IPS-1 was higher in CHB and AHB than in healthy ($P < 0.05$). The IFN-β expression also decreased in CHB than healthy ($P < 0.05$).

Conclusions: Our result showed that the expression of RIG-I and IPS-1 in MoDC were abnormal with HBV infection, suggesting that RIG-I signaling pathway might be blocked by HBV.The impaired function of MoDC may play a role in HBV infection and chronicity.

OL-101

Preclinical & clinical development of an anti-HBV RNA_i-based therapeutic

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Current therapies for chronic hepatitis B are indefinite and pose significant undesired side effects in patients. There is a clear unmet need for chronic hepatitis B therapies; to shorten treatment times, to prevent selection drug resistance, to add a different mechanism of action to the treatment armamentarium to enhance efficacy of current drugs. Unlike current small molecule HBV therapies, RNAi-based drug product NUCB1000 suppresses not only viral replication but also viral antigen expression. NUCB1000 is designed to be effective against all HBV genotypes and has demonstrated activity against known drug resistant mutants. Because NUCB1000 targets multiple HBV RNA sequences, it is not predicted to select for viable escape mutants over the course of therapy. NUCB1000 is currently undergoing safety evaluations in Phase-1b clinical studies in CHB patients.

The study design of this first-in-man study is in CHB patients with minimal disease. A total of 15 patients will be enrolled in this single dose study with 3 patients in each cohort. When this study is completed the product would have been evaluated for safety over nearly 2 orders of magnitude. At this time, the 1st cohort consisting of 3 patients have been dosed with minimal transient side effects of grade II fever, myalgia, Pharyngodynia. All three patients fully recovered from these early acute symptoms, and the next cohort is currently being enrolled at a dose level 3x that was administered to the initial cohort. The presentation will provide an overview of RNA interference technology, preclinical and clinical development of its eiRNA-based anti-HBV therapeutic.

OL-102

Long-term follow-up of entecavir treated protocol-defined non-responders in rollover study ETV-901

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Background/Aims: Patient management criteria in the entecavir (ETV) nucleoside-naive studies dictated that protocol-defined 'Non-Responders' discontinue study therapy. However, 'Non-Responders' who enrolled in rollover study ETV-901 continued ETV therapy and the follow-up of these patients is reported here.

Methods: 679 nucleoside-naive HBeAg (+)/(-) patients were enrolled and treated with ETV in studies ETV-022/-027. Patients who failed to achieve HBV DNA <0.7 MEq/mL (~700,000 copies/mL) at Week 48 or during Year 2 were classified as protocol-defined 'Non-Responders'. HBV DNA, ALT normalization, and HBV serology were assessed during long-term follow-up in ETV-901 (1 mg ETV).

Results: Thirty out of 679 (<5%) patients treated with ETV in studies ETV-022 and ETV-027 met protocol definition of 'Non-Response'. Among 21 HBeAg(+) protocol-defined 'Non-Responders' who enrolled in ETV-901: 15 achieved HBV DNA <300 copies/mL, 21 achieved ALT <1 × ULN and 7 achieved HBeAg seroconversion. Four patients experienced virologic breakthrough, one of them with a concurrent ALT flare. No patients had evidence of genotypic resistance. No patients discontinued treatment due to adverse events.

Conclusions: Fewer than 5% (30/679) of nucleoside-naive HBeAg(+)/(-) patients treated in ETV-022/-027 met the protocol-defined criteria of 'Non-Response'. Most 'Non-Responders' who continued to receive ETV treatment in roll-over study ETV-901 achieved HBV DNA <300 copies/mL and ALT normalization. Although 4 patients experienced virologic breakthrough, no patients had evidence of genotypic resistance to ETV. Treatment compliance should be evaluated in patients with non-response.

OL-103

Three-year continuous entecavir treatment in Chinese patients who had previously failed lamivudine: results from studies ETV-056 and -050

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Background/Aims: In study ETV-056, 48 weeks of entecavir treatment of Chinese patients who previously failed lamivudine therapy resulted in 27% of patients achieving HBV-DNA <300 copies/mL. 138 entecavir-treated patients from study ETV-056 enrolled in rollover study ETV-050. We present efficacy and safety results in a cohort of patients from ETV studies -056 and -050 who received 3 years of therapy with ETV.

Methods: This cohort consists of patients who had previously failed LVD treatment and completed at least 36 weeks of ETV 1 mg in Study-056 and enrolled into ETV-050. Analysis of this cohort allowed evaluation of virologic, serologic and ALT outcomes after 3 years of continuous ETV treatment in patients with available samples at week 144.

Results: The 056/050 ETV-continuous cohort consists of 138 patients. Efficacy parameters for patients were examined. After 3 years of therapy, among patients with available samples, 54/98 (55%) achieved HBV DNA <300 copies/mL; 30/46 (65%) achieved ALT ≤1 x ULN; 7/95 (7%) experienced HBeAg loss; and 2/95 (2%) experienced HBeAg seroconversion. By protocol design, most patients with consolidated response at 48 weeks or during Year 2 discontinued study therapy and were not included in cohort. Numbers and proportions of patients with HBeAg loss and HBeAg seroconversion represent additional patients achieving these endpoints during ETV-050.
Conclusions: In this cohort of Chinese patients who previously failed lamivudine, continuous treatment with 3 years of ETV resulted in 55% achieving undetectable HBV DNA and 65% ALT normalization. The safety profile was consistent with the previously reported experience.

OL-104

Personalized medicine: which chronic hepatitis B patients should treat with interferon?

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In hepatitis B e antigen (HBeAg)-positive patients, the HBeAg seroconversion rate reaches 32% when treated with the pegylated interferon alfa (Peg-IFN) [1]. There are many other anti-viral drugs available for treating HBV infected patients. To find which patients should be treated with interferon, we have conducted a study to screen for genes and single nucleotide polymorphisms (SNPs) which influence the efficacy of interferon therapy among the Asian populations[2]. Based on the results of such a study, we have developed a pharmacogenomics algorism using human genotypes to predict the efficacy of HBeAg-positive patients with Peg-IFN treatments.
The experiment has been designed based on our previous experience with HCV project[3, 4]. In the current study, more than 200 SNPs from 25 candidate genes were genotyped with samples collected from 200 Peg-IFN treated CHB patients. After a serious of computer-aided analysis, a total of 30 polymorphisms were found to be strongly associated with responders and non-responders. A panel of SNPs was further selected to predict the treatment outcomes.
Our investigation demonstrated the potential use of pharmacogenomics approach: by forecasting drug efficacy one can select proper therapy prior to treatment. In the case with CHB patients, this genetic predisposition factors can be used to select IFN or anti-viral drugs.

OL-105

Baseline characteristics and early response predictors of therapeutic outcomes in telbivudine- or lamivudine-treated HBeAg-Positive chronic hepatitis B patients

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Background: Prediction of long-term treatment outcomes, based on baseline parameters and early treatment response, and choice of initial therapy may help optimise patient care for chronic hepatitis B (CHB).
Methods: The GLOBE trial randomised 1367 CHB patients (921 HBeAg-positive patients) to telbivudine (600 mg/day) or lamivudine (100 mg/day) for 104 weeks. Results with telbivudine and lamivudine were compared, and effects of baseline parameters and early viral suppression were assessed by multivariate analyses.
Results: In both HBeAg-positive treatment groups, baseline HBV DNA and ALT levels were significant predictors of Week 24 viral response and Week 104 outcomes, while Week 24 PCR-negativity was the strongest predictor of Week 104 outcomes. Among patients with baseline ALT ≥2xULN and HBV DNA <9 $\log_{10}$ copies/mL (guideline-eligible with commonly-encountered baseline DNA levels), 71% of telbivudine-treated and 54% of lamivudine-treated patients were PCR-negative at Week 24 (P=0.022). At Week 104, among patients with ALT ≥2xULN and HBV DNA <9 $\log_{10}$ copies/mL at baseline who achieved Week 24 PCR-negativity, telbivudine trended toward higher rates of PCR-negativity (89% vs 76%) and seroconversion (52% vs 46%), with less viral breakthrough (3.6% vs 12%), compared with lamivudine.
Conclusions: Week 24 PCR-negativity, low baseline HBV DNA, and high baseline ALT were predictive of 2-year treatment outcomes in HBeAg-positive patients. Long-term outcomes were better with telbivudine than lamivudine, including HBeAg-positive patients with baseline ALT ≥2xULN and HBV DNA <9 $\log_{10}$ copies/mL who achieved Week 24 PCR-negativity, suggesting that baseline and on-treatment characteristics and initial choice of therapy can help optimise patient management.

OL-106

Entecavir (ETV) results in higher HBV DNA reduction versus adefovir (ADV) in antiviral-Naive HBeAg(+) adults with High HBV DNA: 96 week results (E.A.R.L.Y. Study)

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Background: Previous results demonstrated superior early HBV DNA reduction for ETV versus ADV at Weeks 12 and 48. We present Week 96 results from this randomized, open-label study in HBeAg(+), antiviral-naive CHB patients.
Methods: Sixty-nine patients were randomized 1:1 to either ETV (0.5 mg) or ADV (10 mg) QD for 52 weeks. Protocol-defined 'Responders' (HBe seroconversion and HBVDNA <10,000 copies/mL for 24 weeks with last HBV DNA <300 copies/mL) discontinued from therapy and were observed off-treatment. Patients who discontinued due to inadequate therapeutic response could enter a long-term ETV rollover study. All other patients continued in a second year. Serum HBV DNA by PCR (COBAS Amplicor), HBV serology and safety laboratories were obtained through Week 96.
Results: Twenty-nine ETV- and 20 ADV-treated patients were treated in Year 2 and baseline mean viral load for was similar for the two groups (10.40 and 9.93 $\log_{10}$ copies/mL, respectively). ETV treatment resulted in a significantly greater mean viral load reduction compared to baseline (-7.82 versus -5.96 $\log_{10}$ copies/mL), and a higher proportion of patients achieving HBV DNA <300 copies/mL (79% versus 50%). ALT normalization and seroconversion occurred in comparable proportions of patients, however total study discontinuations were higher in the ADV arm (44%) versus ETV (12%).
Conclusion: ETV treatment resulted in a greater decrease from baseline in HBV DNA than ADV through Week 96, with more patients achieving undetectable HBV DNA. Fewer ADV-patients were treated through 96 weeks due to a higher proportion of discontinuations. Both treatments were well tolerated.

OL-107

Entecavir at five years shows long-term maintenance of high genetic barrier to hepatitis B virus resistance

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Background/Aims: Entecavir (ETV) provides both potent viral suppression and a high genetic barrier to resistance, requiring ≥3 resistance substitutions. As a result, in nucleoside-naive patients, ETV resistance (ETVr) was rare through 4 years. The genetic barrier in lamivudine (LVD)-refractory patients was reduced.
Methods: All patients receiving continuous therapy in phase II/III trials were monitored for resistance through year 5. Detectable patient HBV DNA (≥300 copies/mL) was sequenced in each year. Cumulative probabilities of resistance were determined through year 5.
Results: In years 1 through 5, respectively, 663, 278, 149, 120 and 108 nucleoside-naive patients were treated and monitored, with 93% in year 5 having HBV DNA <300 copies/mL. No patients with year 5 detectable virus showed emerging ETVr at T184, S202 or M250 ± LVD resistance (LVDr) M204I/V±L180M. The cumulative probability of genotypic ETVr remained 1.2% through 5 years. Among

LVD-refractory patients treated with ETV, 187, 146, 80, 53 and 33 were monitored in years 1 through 5, respectively. The cumulative probabilities of genotypic ETVr at years 1 through 5 were 6%, 15%, 36%, 46% and 51%, respectively, and virologic breakthrough with ETVr was 43% through year 5. Among 68 LVD-refractory patients who achieved undetectable HBV DNA on ETV, 1 subsequently developed ETVr with virologic breakthrough.
Conclusions: ETVr remains rare (1.2%) in nucleoside-naive patients through 5 years. LVDr patients have a reduced barrier to ETV resistance and may benefit from combination therapy.

OL-108
Baseline parameters and early response predict therapeutic outcomes in HBeAg-negative chronic hepatitis B patients treated with telbivudine or lamivudine
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Background: In patients with chronic hepatitis B (CHB), long-term treatment outcomes may be optimised through informed selection of initial therapy and individualisation of patient management based on baseline characteristics and early treatment response.
Methods: In the GLOBE study, 1367 patients with CHB (446 HBeAg-negative patients) were randomised to telbivudine (600 mg/day) or lamivudine (100 mg/day) for 104 weeks. Multivariate analyses assessed the effects of baseline and on-treatment variables on Week 104 outcomes, and outcomes with telbivudine and lamivudine were compared.
Results: In both HBeAg-negative treatment groups, superior Week 104 outcomes were achieved in the subgroup with baseline HBV DNA <7 $\log_{10}$ copies/mL (telbivudine n=91; lamivudine n=103). In this subgroup, significantly more telbivudine recipients were PCR-negative at Week 104, compared with lamivudine (89% vs 67%, P<0.001), and resistance was significantly lower (3% vs 20%; P<0.001). In multivariate analyses, Week 24 PCR-negativity was the strongest predictor of Week 104 outcomes, and more telbivudine recipients with baseline HBV DNA <7 $\log_{10}$ copies/mL became PCR-negative at Week 24 compared with lamivudine (95% vs 81%, P=0.0039). Among patients with baseline HBV DNA <7 $\log_{10}$ copies/mL who achieved Week 24 PCR-negativity, telbivudine demonstrated greater maintenance of PCR-negativity (91% vs 72%, P=0.0020) and less resistance (2% vs 17%, P=0.0012) through Week 104, compared with lamivudine.
Conclusions: Week 24 PCR-negativity and baseline HBV DNA were predictive of Week 104 outcomes in HBeAg-negative patients. Telbivudine demonstrated superior long-term outcomes compared with lamivudine in HBeAg-negative patients, including those with baseline HBV DNA <7 $\log_{10}$ copies/mL who achieved Week 24 PCR-negativity.

OL-109
In vitro cross-resistance profile of telbivudine
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Several nucleoside (entecavir, lamivudine and telbivudine) and nucleotide (adefovir) analog inhibitors are approved for the treatment of hepatitis B virus (HBV) infection and several others, including tenofovir, are in clinical development.
To investigate the resistance profiles of these agents, HepG2 cells were stably transfected with wild-type or mutant HBV genomes bearing mutations known to confer resistance to each of the different agents. The activity of the viral polymerase was assayed after drug treatment. Against HBV genomes bearing the known telbivudine resistance mutations, M204I and M204I/L80I, telbivudine, lamivudine and entecavir lost 353 to >1000 fold activity whereas adefovir and tenofovir exhibited no more than 3-5 fold change. Against the A194T mutant associated with tenofovir resistance, telbivudine remained fully active. Against HBV cell lines expressing the adefovir resistance mutations N236T and A181V, telbuvidine remained active as shown by respective fold-changes of only 0.5 and 1.0 to 3.5 (depending on the test cell line). In contrast, adefovir was around 3.9 fold less active against the N236T mutant and 0.5 to 3.8 fold less active against the A181V mutant. Overall, these results indicate that nucleoside and nucleotide drugs have different cross-resistance profiles, suggesting that the addition of telbivudine to adefovir in patients who developed adefovir resistance might be an effective treatment. *In vitro* studies demonstrating that telbivudine and adefovir give enhanced activity against HBV when used in combination, together with recent clinical studies, provide additional support for the use of nucleoside/nucleotides combinations.

OL-110
Hepatitis B surface antigen-pulsed dendritic cells for treating patients with chronic hepatitis B: A phase I safety and immunogenecity study
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Several preclinical studies have shown the therapeutic utility of antigen-pulsed DCs in animal models of chronic hepatitis B virus (HBV) infection, however, clinical trial with antigen-pulsed DCs have rarely been conducted in patients with chronic hepatitis B (CHB). Here, we have reported about production, treatment schedule, safety and immunogenecity of hepatitis B surface antigen (HBsAg)-pulsed DCs in CHB patients.
DCs were enriched from six patients with CHB by culturing adherent populations of peripheral blood mononuclear cells with human grade and endotoxin-free cytokines, and growth factors for 7 days. HBsAg-pulsed DCs were prepared by culturing human blood DCs with a human consumable hepatitis B vaccine containing 10 μg of HBsAg. Immunogenecity of HBsAg-pulsed DCs were assessed in vitro form their expression of HLA DR & CD86, and production of proinflammatory cytokines. Five million autologous HBsAg-pulsed DCs were injected interdermally, twice to each patient. All patients were followed up for 24 months. All patients were periodically checked for different parameters of safety and immune modulatory capacities of HBsAg-pulsed DCs.
No patients exhibited any abnormality in physical, biochemical and immunological parameters including liver and kidney function tests during the follow up period. Administration of HBsAg-pulsed DCs induced detectable levels of anti-HBs in 2 patients and HBsAg-specific lymphocytes in 3 patients.
This study provides the ethical and scientific basis of using HBsAg-pulsed DCs for treating patients with CHB. The concept of this study may be used for development of therapeutic vaccines against chronichepatitis C virus infection and liver cancer.

OL-111
Telbivudine is a cost-effective first-line oral treatment of patients with chronic hepatitis B (HBeAg+) in the UK
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Objectives.
To estimate the most cost-effective oral treatment strategy for patients in the UK with positive chronic hepatitis B. (CHB)
Methods: Using data from the GLOBE study and published adefovir dipivoxil (ADV) data, a seroconversion model was constructed to simulate the progression of chronic hepatitis B (HBeAg+) and associated complications in a cohort of hypothetical patients. Eight treatment strategies were assessed: telbivudine followed by ADV; lamivudine followed by ADV; ADV followed by telbivudine; ADV followed by lamivudine; telbivudine alone; lamivudine alone; ADV alone and best supportive care (BSC) alone. Health states included were cured, inactive carrier, chronic hepatitis B, compensated cirrhosis, decompensated cirrhosis, hepatocellular carcinoma and death. Treatment was withdrawn for patients who were cured, and after 6 months for those who became inactive carriers. The time horizon was that of patient lifetime, with only direct medical costs considered. Costs and utilities were extracted from literature reviews and discounted at 3.5% per annum. Probabilistic sensitivity analyses were conducted and net benefit calculated, using recommended NICE thresholds of £20,000 and £30,000 per QALY gained.[1]
Results: At a threshold of £20,000 per QALY, telbivudine alone was the most cost-effective strategy. When the threshold was increased to £30,000, telbivudine followed by ADV became most cost-effective.
Conclusions: For the strategies considered, and regardless of which cost per QALY threshold was employed, the most cost-effective first-line oral treatment for patients with positive CHB in the UK was telbivudine.
[1] NICE (2004). Guide to the methods of technology appraisal.

OL-112
Efficacy, safety and resistance of entecavir (ETV) following three years of treatment in Japanese patients with lamivudine

(LVD)-refractory chronic hepatitis B

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Background: LVD treatment is associated with the rapid development of resistance and the subsequent loss of clinical benefit. LVD-refractory Japanese patients who completed 52 weeks of ETV treatment in Phase II study ETV-052 (ETV 0.5mg and 1mg) could enroll in an open-label rollover study (ETV-060). In these patients, the efficacy, safety and resistance profile of 3 years treatment with ETV 1 mg daily were evaluated.

Methods: Ninety-eight percent (42/43) of LVD-refractory patients who received ETV 1 mg in ETV-052 entered ETV-060 without a treatment gap. HBV DNA by PCR, histological improvement, ALT, and safety were evaluated. HBV DNA sequencing was performed on all patients whose HBV DNA remained detectable by PCR (≥400 copies/mL).

Results: Thirty-five of 43 patients were treated for a minimum of 148 weeks from baseline. The proportion of patients achieving HBV DNA <400 copies/mL was 54% (19/35) at Week 148. Histological improvement was observed in 81% (13/16) of patients with evaluable repeat biopsies. ALT normalization occurred in 84% (27/32) of patients at Week 148. Three patients discontinued ETV due to adverse events. Also 96% (81/84) of all ETV-052/060 were monitored for resistance. Genotypic ETV resistance was identified in 31 patients. In patients who received ETV 1mg from the beginning, the 3-year cumulative probability of ETV resistance was 33%.

Conclusions: Long-term treatment of LVD-refractory patients with ETV 1mg daily resulted in continued clinical improvement. The pattern of resistance in this study was consistent with worldwide studies. ETV was well tolerated through 3 years of treatment.

OL-113

Economic evaluation of entecavir and telbivudine vs lamivudine for nucleos(t)ide naive HBeAg positive patients with chronic hepatitis B in China

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Background: CHB treatment puts high economic burden on the Chinese health care system due to long treatment duration and high treatment cost.

Objectives: To evaluate the incremental cost-effectiveness of Entecavir and Telbivudine vs. Lamivudine for naive HBeAg positive CHB patients based on two China phase III trials [1,2].

Methods: The evaluation was performed from the Chinese societal perspective, using public drug prices. Treatment cost include one year study drug cost and additional half year cost for resistance treatment with Adefovir. Since resistance data in ETV study is unavailable, rates were assumed as 0% for Entecavir and 14.7% for LAM.

Results: The results of this indirect drug comparison are expressed as incremental cost per 1% additional ALT normalization, HBV DNA undetectable and e-seroconversion.

Conclusion: From complete response perspective, Telbivudine has a better cost-effectiveness ratio compared to Entecavir for naive HBeAg positive CHB patients.

Table 1 Efficacy Data of Entecavir and Telbivudine Trials and Economic Evaluation of Entecavir and Telbivudine vs Lamivudine

	ETV trial[1]		LDT trial[2]	
	ETV n=258	LAM n=263	LDT n=147	LAM n=143
Percentage of HBV DNA <300 copies/mL(PCR)	74%	38%	67%	38%
HBeAg seroconversion	15%	18%	25%	18%
ALT normalization	89%	78%	87%	75%
Resistance	0[3]	14.7%[3]	7.5%	14.7%
One year study drug cost	14235	5840	8760	5840
Additional half year cost for ADV treatment	0	563.4	287.4	563.4
Total annual cost	14235	6403.4	9047.4	6403.4
Incremental cost /% DNA undetectable vs LAM	21754.5		9117.4	
Incremental cost /% e-seroconversion vs LAM	dominated (-261054.1)		37772.3	
Incremental cost /% ALT normalization vs LAM	71196.6		22033.8	

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[2] Hou JL, Yin YK, Xu DZ, et al. Telbivudine Versus Lamivudine in Chinese Patients with Chronic Hepatitis B: Results at 1 Year of a Randomized, Double-Blind Trial. Hepatology 2008;47:447-454.

[3] Assumption: LAM resistance is similar to what reported in LDT trial, which is also align with China Chronic hepatitis B guideline.

Note: Chinese Yuan (RMB) is used for calculation.

OL-114

Increased frequency of regulatory T cells is associated with liver injury in patients with chronic hepatitis B

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Background: Recent studies have suggested that Regulatory T (Treg) cells may be involved in immune regulation in patients with chronic hepatitis B (CHB), but their correlation with the level of serum ALT and HBV DNA is controversial. This study attempted to characterize Treg cells in peripheral blood mononuclear cells (PBMC) in CHB patients in different phases, and to explore the relationships between the Treg cells and serum ALT or HBV DNA level.

Methods: The frequency and phenotypic characteristics of Treg cells in PBMC from 22 CHB patients, including 9 in group 1 (immune-tolerant phase, ALT<30U/L, HBsAg and HBeAg positive, HBV DNA>107 copies/ml) and 13 in group 2 (immune clearance phase, ALT>100U/L, HBsAg and HBeAg positive, HBV DNA>107 copies/ml), were analyzed through flow cytometry. The frequency of Treg cells was defined as the percentage of CD4+CD14-CD25+Foxp3+ T cells in total CD4+CD14- T cells.

Results: The frequency of Treg cells was significantly increased in PBMC from CHB patients in group 2 as compared to those in group 1 (mean, 2.03±0.74% vs 1.16±0.37%, $P<0.05$, Student's T test) and a significant correlation was observed between the frequency of Treg cells and serum ALT level ($r=0.531$, $P<0.05$, Spearman correlation analysis). However, HBV DNA level had no significant association with Treg cell frequency ($r=0.112$, $P>0.05$, Spearman correlation analysis).

Conclusions: The frequency of Treg cells in CHB patients in immune clearance phase was significantly higher than that in immune tolerance phase, and increased Treg cells were associated with liver injury.

Acknowledgment: This work was supported by the grants from the 973 National Basic Research Program of China (2007CB512800), RGC (HKU 7679/06M) to Prof. George K.K. Lau and Cheng Si Yuan China-International Hepatitis Research Foundation.

OL-115

Rapid quantitation of adefovir-resistant mutants in adefovir treated patients with chronic hepatitis B virus infection

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Background: Rapid and quantitative methods to determine the percentage of adefovir-resistant mutants in total HBV are important during adefovir therapy.

Methods: We established a quantitative real-time PCR method with selective primers and TaqMan probe.The percentage was calculated between threshold cycle number (ΔCt) of mutant and control reactions. Clones containing different adefovir variants were diluted and tested.

30 serum samples were analyzed with this method and compared with DNA sequencing.

Results: As little as 1% mutant plasmids in 10^6-10^9 copies/ml of wild-type plasmids were detected. 9 of the patients had mutants with percentages of 5-100%. Real-time PCR detected mutants with percentages as few as 5-20%, which were concordant with subclone sequencing.

Conclusion: This real-time PCR is a rapid, sensitive method for relative quantitation of adefovir mutants of HBV.

OL-116

Virological and biochemical response in patients with HBeAg-negative chronic hepatitis B treated with peginterferon alfa-2a (40KD) with or without lamivudine: results of 4-year follow-up

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Background: Virological and biochemical response rates and HBsAg clearance 3 years post-treatment in HBeAg-negative CHB patients were significantly higher for peginterferon alfa-2a (40KD) [PEGASYS] for 48 weeks ± lamivudine versus lamivudine alone.

Methods: Patients received 180µg PEGASYS plus placebo (n=177), 180µg PEGASYS plus 100mg lamivudine (n=179), or 100mg lamivudine (n=181) and were assessed 6 months post-treatment. 230/356 patients treated with PEGASYS ± lamivudine and 85/181 treated with lamivudine alone participated in a long-term roll-over observational study.

Results: 4 years post-treatment 24% of PEGASYS-treated patients (55/230) had HBV DNA <20,000 copies/mL (~4,000 IU/mL). Sustainability of HBV DNA <20,000 copies/mL for patients with available data through post-treatment years 1 to 4 was 80%. 38/230 (17%) of PEGASYS-treated patients had HBV DNA <400 copies/mL versus 6/85 (7%) for lamivudine alone (p=0.032). 63/230 (27%) of PEGASYS-treated patients had ALT normal vs 14/85 (16%) for lamivudine alone (p=0.045). HBsAg clearance in PEGASYS-treated patients increased to 11% (25/230) but reached 2% (2/85) in patients treated with lamivudine alone (p=0.017). 19/54 (35%) of PEGASYS-treated patients with HBV DNA levels ≤400 cp/mL 24 weeks post-treatment cleared HBsAg vs only 6 patients with HBV DNA above this level (range: 569–327,000 copies/mL). None of the 7 lamivudine-treated patients with HBV DNA ≤400 copies/mL 24 weeks post-treatment cleared HBsAg.

Conclusions: 1-year of PEGASYS provided durable HBV DNA suppression over 4-year follow-up in around 1/4 patients with HBeAg-negative CHB. This, together with its ability to induce during follow-up increasing rates of HBsAg clearance, supports its use as first-line therapy for HBeAg-negative CHB.

OL-117

Baseline ALT levels predict virologic response for adefovir dipivoxil treatment patients with chronic hepatitis B

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Background: This report is to describe Chinese HBeAg+ subjects with different ALT during ADV monotherapy up to four years. The results may provide useful information for predicting response to ADV treatment.

Methods: We retrospectively analyzed 240 HBeAg+CHB patients, serum ALT levels over the upper limit of normal (ULN) 1-2times，2-5times and more than 5times, treated with ADV 10 mg/d up to 208 weeks. They were followed up every 12 weeks. If they had HBeAg/anti-HBe seroconversion, HBV DNA< 300copies/ml and normal liver function then continued treated with ADV 6 months, and follow up till 208 weeks.

Results: In patient with baseline ALT 2-5xULN the rates of HBeAg loss was 21%, 37%and 42%up to 104w, 156w and 208w, respectively. Whereas, ALT >5xULN, HBeAg loss was 30%,48%and respectively, in ALT<2xULN group they are low (P<0.05). Baseline ALT 2-5xULN HBeAg/anti-HBe seroconversion rates was 15%, 21% and 23% up to 104w, 156w, 208w, respectively. ALT >5xULN patients, seroconversion rates was 28%, 30%, 38% at weeks 104,156,208, respectively. They are higher than ALT<2xULN group (p<0.05).

Conclusion: HBeAg loss and seroconversion rates were significantly correlated with baseline ALT levels, long-term therapy could be obtained good reaction.

ALT(Baseline)ULN	HBeAg loss			HBeAg loss and HBeAg/Anti HBe		
	104W	156W	208W	104W	156W	208W
1-2	12%(8/67)	20%(13/65)	26%(16/61)	7% (5/67)	14%(9/65)	10%(6/61)
2-5	21%(20/95)	37%(33/90)	42%(36/86)	15% 14/95)	21%(19/90)	23%(20/86)
>5	30%(14/46)	48%(22/46)	60%(27/45)	28%(13/46)	30%(14/46)	38%(17/45)

Free Paper Presentation – HCV

OL-118

Differential dysregulation of T and B lymphocyte signaling by hepatitis C virus

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Hepatitis C virus (HCV) infection is characterized by a strong propensity toward chronicity, autoimmunity, and lymphomagenesis, suggesting a role for lymphocyte dysregulation during persistent viral infection. We have previously reported that HCV core protein inhibits T cell functions through interaction with a complement receptor-gC1qR. Our recent data shown that while T cell function is suppressed during chronic HCV infection, B cells often exhibit activation phenotype and clonal expansion. Importantly, we further demonstrated that HCV infection alters T and B cell functions through induction of negative modulators, programmed death-1 (PD-1) and suppressor of cytokine signaling-1 (SOCS-1). Interestingly, exposure to HCV core protein leads to a differential regulation of these key immunemodulators (PD-1/SOCS-1) in T and B lymphocytes from healthy subjects, supporting a role for this protein in the immunodysregulation observed in chronic HCV-infected individuals. HCV core /gC1qR interaction decreased CD69 expression, interferon-γ production in T cells; but increased CD69, CD86 (B7-2), CD154 (CD40L), CD195 (CCR5), immunoglobulin (IgG/IgM) expression and cell proliferation in B cells. Finally, we observed up-regulation of SOCS-1 accompanied by down-regulation of signal transducer and activator of transcription-1 (STAT-1) phosphorylation in T cells in response to HCV core protein, with the opposite effect observed in HCV core-treated B cells. This study, demonstrating a differential regulation of T and B lymphocyte signaling by HCV, represents a novel and perhaps common pathway by which a virus usurps host machinery to facilitate persistence and autoimmune phenomena.

OL-119

Pharmaceutical compositions based on HCV-like particle that stimulates both interferon-α and B cells

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Novel recombinant non-infectious non-replicating virus-like particle ISVAC® built of structural proteins of HCV, is found to strongly stimulate serum interferon-α in whole human blood and in healthy human volunteers, as well as B cells in murine spleen. Levels of both stimulations exceed those of respective known analogues: interferon inducers such as poly I:C and CpG ODN-A, as well as B cell mitogens including CpG ODN-B. These results suggest that ISVAC® stimulates Toll-receptors (TLR). This hypothesis correlates with current finding that TLR9-driven interferon induction in plasmacytoid dendritic cells requires CpG ODN being delivered by nanoparticle, hence we propose that ISVAC® stimulates TLRs by delivering to them some nucleic acids incorporated during its production or travel through tissues. Pharmaceutical composition based on ISVAC® displayed perfect safety profile in formal rodent toxicology as well as in human stem cell culture studies and healthy human volunteers. Thus, ISVAC® appears to be more physiological analogue of CPG-ODNs and the first representative of the new class of TLR stimulators providing novel powerful tool for fundamental research of both type-I-interferon and B cell stimulation and action as well as for respective clinical and pharmaceutical applications that include Hepatitis C, multiple sclerosis, asthma, and other numerous diseases respondng to interferon or B cell simulation.

OL-120

In vitro antiviral activity and preclinical profile of TMC435350, a novel HCV NS3/4A serine protease inhibitor

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Hepatitis C NS3/4A serine protease inhibitors have shown potential in clinical trials either as monotherapy or in combination with PEG-Interferon and ribavirin therapy. TMC435350 is a novel and potent HCV NS3/4A protease inhibitor currently undergoing clinical evaluation.

TMC435350 exhibited K_i values of 0.5 and 0.4 nM for enzyme subtypes 1a (H77) and 1b (con1) respectively and more than 1000-fold less activity against 23 other human proteases tested. In the subgenomic genotype 1b replicon model, TMC435350 had an EC_{50} of 8 nM and a selectivity index (SI) of > 2000. Combining TMC435350 with different HCV inhibitor classes including Interferon, ribavirin and polymerase inhibitors further increased the reduction in HCV RNA in an additive to synergistic manner, and further reduced the emergence of resistant replicon colonies. After a single oral administration of TMC435350 at 40 mg/kg in rats, the mean peak plasma concentration (C_{max}) was 1430 ng/mL which was observed at 2 hours post-dose (t_{max}) and showed an absolute bioavailability of 44%. In rats, TMC435350 was found to be extensively distributed to the liver and small- and large intestines (tissue/plasma ratios > 35). Concentrations in other organs were similar to plasma. Notably, TMC435350 was still quantifiable in the liver tissue up to 31 hours post-dosing.

TMC435350 is a novel, potent and specific HCV protease inhibitor, with good oral bioavailability and a favorable liver distribution. In addition, in vitro studies support the potential use of TMC435350 in combination with other HCV inhibitors. TMC435350 is now in phase II clinical trials.

OL-121

Progression to cirrhosis in hepatitis C is associated with cognate inhibitory KIR and HLA-C genes

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Background and Aim: Natural Killer (NK) cells likely play pivotal roles in HCV immunity [(1)]. They are regulated by polymorphic repertoires of activating and inhibitory receptors specific for hepatic HLA class I ligands. Homozygosity for the weak inhibitory NK cell receptor KIR2DL3 and its HLA-C1 ligand confers protection against low-inoculum HCV infection [(1)], presumably by permitting strong activation. Activation of NK cells results in cytolytic function and cytokine release, which in the liver potentially promote fibrosis. We therefore investigated immunogenetic predisposition to NK cell activation and progressive liver disease in patients who fail to clear acute HCV.

Methods: HCV-related cirrhotic patients were identified by biopsy and/or clinical criteria.

Genomic DNA was extracted from PBMC.

Genotyping: SSP-PCR to distinguish 16 KIR genes/pseudogenes.

HLA-C typing: Nested-PCR developed to distinguish HLA-C1 from HLA-C2 alleles.

Statistical analyses: Chi-square tests from 2x2 contingency tables.

Results: 205 patients with HCV-related cirrhosis were genotyped for KIR and HLA-C: 147 (71.7%) Caucasians, 30 (14.6%) Hispanics, 15 (7.3%) African Americans, 9 (4.4%) Asians and 4 (2%) were of other ethnicities. Individual KIR and ligand (HLA) gene frequencies in cirrhotic patients were equivalent with those in a chronic cohort [(1)] and in a separate control cohort[(2)].

In cirrhotics, the frequency of HLA-C1C1 (42 %) and of KIR2DL3/2DL3: HLA-C1C1 (20.5%), were significantly higher than among 685 people with persistent HCV infection [(1)] (29.9% and 12.3% respectively): $p = 0.0012$ and p=0.003 respectively.

Conclusion: People with hepatitis C possessing the compound genotype KIR2DL3/2DL3:HLA-C1C1 who fail to clear acute infection are more likely to develop cirrhosis, potentially promoted by increased NK cell activation.

References

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OL-122

Emodin inihibits cell invasiveness induced by HCV

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There are several clinical and basic reports about intrahepatic spread of transformed cells during hepatocellular carcinoma course (HCC). Hepatitis C virus (HCV) seems to be the major causative viral agent of cirrhosis and hepatocarcinoma. But systemic chemotherapy for unresectable or metastatic HCC are quite ineffective.The metastatic capability is related to the modulation of cell adhesion and motility and since the molecular and cellular mechanisms controlling these events are not completely understood we decided to analyze them in depth in HCV-related HCC.
HepG2 stable polyclones expressing both HCV-wt and HCV core protein induced paxillin and beta1-integrin expression levels increase; but no changes were observed in alpha-actinin expression. IF assay showed a spread beta1-integrin distribution in the cytosol and alpha actinin delocalization from the cytosol to the perinuclear region, in HCV-wt polyclones. HCV core protein silencing by a specific siRNA completely abrogated HCV-related effects. In addition, Focal Adhesion Kinases (FAK) expression and activity was increased in HCV wt polyclones and HCV core protein; HCV core siRNA re-estabilished FAK basal levels in these cells.
Interestingly, FAK siRNA targeting only partially reverted the HCV focal adhesion deregulation, whereas emodin completely abrogated it.
Emodin counteracting HCV related adhesion molecules alterations could be considered as a valid alternative to the therapeutic approaches to date available.

OL-123

Mouse model for hepatitis C virus replication

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A reliable small animal model is needed to test the success of the intracellular immunization strategy against hepatitis C virus in vivo. We prepared interferon sensitive Huh-7 cell line replicating green fluorescence-tagged HCV sub-genomic RNA. Mouse adapted GFP replicon cell clone was isolated from subcutaneous tumors and serially passaged in SCID/bg mice followed by selection with G-418. Replicon cells were implanted subcutaneously into gamma irradiated SCID mice to form a subcutaneous tumor. This model was validated by subcutaneous injection of interferon alpha daily up to 2 weeks. Highly tumorgenic sub clone of original interferon sensitive replicon was isolated that express high levels of GFP after four passages in SCID mice. These replicon cells developed subcutaneous tumors in gamma irradiated SCID/bg mice in approximately two weeks. High levels of HCV replication in the Xenograft tumors were confirmed by detection of HCV positive strand RNA by ribonuclease protection assay and GFP expression by fluorescence microscopy. Interferon alpha treatment completely cleared HCV RNA replication and expression in the subcutaneous tumors within two weeks. We showed that HCV replication in the subcutaneous tumors can be effectively inhibited by interferon alpha suggesting that this mouse model can be used to test novel therapeutics against HCV.

OL-124

The role of the ISG15/USP18 pathway in modulating interferon sensitivity in hepatitis C virus infection

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Background and Aims: Treatment of chronic hepatitis C virus (HCV) infection remains problematic: pegylated IFN/ribavirin eradicates the virus in only 50% of patients. We recently found that upregulation of the ISG15 ubiquitin-like protein, and of USP18, an ISG15 protease, correlates with treatment non-response (Chen, Gastroenterology 2005), and that silencing USP18 markedly potentiates the anti-HCV effect of IFN (Randall, Gastroenterology 2006). Whether the effects of USP18 on HCV replication are mediated through ISG15 is unknown, as is the role of ISG15 in HCV viral replication.
Methods: The role of ISG15 in the viral life cycle and IFN antiviral activity was examined using the J6/JFH1 HCV in vitro culture model that reproduces the full viral life cycle . ISG15 conjugation was inhibited with small interfering RNAs (siRNAs) directed against the E1 activating enzyme Ube1L, or increased by plasmid DNA , and the dose response of HCV replication and infectious virus production to IFNα was measured.
Results: Ube1L knockdown abrogated the IFN-dependent ISG15-conjugation of cellular proteins in Huh7.5 cells. Inhibiting ISGylation both decreased baseline HCV infectious particle release and potentiated the effect of IFN by 3 to 5-fold ($p<0.05$) There was a less marked inhibition of HCV RNA transcription. By contrast, overexpression of ISG15 increased cellular protein ISGylation (western blot), and increased baseline HCV infectious particular production by 6-fold ($p<0.05$) and HCV RNA transcription by 4 fold ($p<0.05$). Since ISG15 can act as a cytokine, we tested the ability of purified ISG15 to modulate IFN responses and found no effect.
Conclusions: The data suggests that while USP18 can potently modulate the IFN-driven anti-HCV response, ISG15 is crucial for full HCV viral replication.

OL-125

Dose selection of albinterferon alfa-2b (alb-IFN) for a phase 3 clinical program

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Albinterferon alfa-2b, a novel, long-acting interferon (IFN) for treatment of chronic hepatitis C (CHC), demonstrated promising antiviral activity and tolerability in preclinical/early clinical studies. The results of a phase 2b, active controlled, open-label, 48-wk, dose-ranging, alb-IFN trial were used to determine dosage regimens for evaluation in ongoing phase 3 studies. 458 IFN treatment-naïve patients with genotype (Gt) 1 CHC were randomized 1:1:1:1 to PEG-IFNα-2a 180μg qwk, or alb-IFN 900μg q2wk, 1200μg q2wk, or 1200μg q4wk, all with weight-based oral ribavirin. Compared with PEG-IFNα-2a (n=114), alb-IFN 900μg (n=118) had a comparable SVR rate (58.5% vs 57.9%; *P*=.93) and early virologic response profile; in adherent patients (≥80% adherence) a higher SVR rate (72% vs 67%; *P*=NS); lower relapse rate (21% vs 29%; *P*=NS) and comparable breakthrough rate; less impaired HRQOL, as measured by SF-36 and missed workdays; and comparable AE-related discontinuation rates. Compared with PEG-IFNα-2a, alb-IFN 1200μg q2wk (n=110) had an improved early response rate, with significantly higher wk2 rate (47% vs 32%; *P*=.02); comparable SVR rate (55.5% vs 57.9%; *P*=.71) despite higher discontinuation rates; and higher SVR rate (71% vs 67%; *P*=NS) in adherent patients. Based on these data, both alb-IFN q2wk dosing regimens are being evaluated in two phase 3 clinical trials in IFN-naïve patients with Gt 1 and Gt 2/3 CHC. Study protocols will reinforce dose-modification strategies to manage tolerability issues.

OL-126

Safety profiles of percutaneous liver biopsy in hemodialysis patients with chronic hepatitis C pre-treated with 1-Deamino-8-D-Arginine vasopressin

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Background/Aims: Percutaneous liver biopsy (PLB) is the gold standard for grading necroinflammation and staging fibrosis in patients with chronic hepatitis C (CHC). Whether the use of 1-deamino-8-D-arginie vasopressin (DDAVP) before PLBs in hemodialysis (HD) patients with CHC has comparable safety profiles to CHC patients with normal renal function has not been evaluated in prospective studies.
Methods: From January 2005 to January 2008**,** 1067 PLBs (258 biopsies in 176 HD patients [Group 1] and 809 in 478 patients with normal renal function [Group 2]) were performed and the biopsy-related complications were prospectively recorded. All HD patients received 0.3 μg/kg body weight DDVAP infusion 30 to 60 minutes before the biopsies, and all patients received two passes of automatic-needle biopsies under ultrasonography guidance. The primary endpoint was biopsy-related serious hemorrhage rate, defined as fatal and non-fatal admission rates from hemorrhage within 2 weeks of biopsies. The secondary endpoint was mild hemorrhage rate without admission.
Results: The serious hemorrhage rates were comparable between the two groups (0% vs. 0.5% in Group 1 and 2, respectively, $p = 0.58$). The mild hemorrhage rates were also comparable (19.0% vs. 17.7% in Group 1 and 2, respectively, $p = 0.64$). In addition, only 4 (1.6%) and 2 (0.8%) of the 258 biopsies in HD patients had transient facial flushing and mild headache during the DDAVP infusion.
Conclusions: Pre-treatment of DDAVP is safe in HD patients with chronic hepatitis C receiving PLBs, and its use can achieve comparable safety profiles to CHC patients with normal renal function.

Poster Session – Acute Liver Failure

PP-001

Suppression of immune-mediated liver injury after vaccination with attenuated pathogenic cells

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Cell vaccination via immunization with attenuated pathogenic cells is an effective preventive method that has been successfully applied in several animal models of inflammatory or autoimmune diseases. Concanavalin A (Con A)-induced hepatitis (CIH) is a commonly used experimental model to study immune-mediated liver injury. Multiple cell types including T lymphocytes, macrophages and neutrophils have been found to be involved in the pathogenesis of CIH. In this study, we used attenuated spleen lymphocytes or peripheral blood lymphocytes as vaccines to investigate whether they could induce protective immune responses to prevent mice from developing CIH. We found that mice receiving such vaccination before CIH induction developed much milder diseases, exhibited a lower level of alanine aminotransferase (ALT) released into their plasma and had less inflammatory lesions in their livers. Such CIH-suppression is dose- and frequency-dependent. The suppressive effect was associated with inhibition of several major inflammatory mediators, pro-inflammatory cytokines and chemokines.

PP-002

Vasoactive intestinal peptide protects mice from immune-mediated liver damage

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Vasoactive intestinal peptide(VIP) has a brand range of biologic actions and is well characterized as an endogenous anti-inflammatory neuropeptide. In this study, we aimed to investigate the effects of VIP administration on Con A-induced hepatitis (CIH) in mice, a widely used experiment model of immune-mediated liver injury. Mice were treated with VIP before CIH induction. Alanine aminotransferase (ALT) levels in plasma, inflammatory infiltration and hepatocyte apoptosis in the liver were measured, and potential therapeutic mechanisms were elucidated. Compared with control mice treated with vehicle solutions, mice pretreated with VIP exhibited much lower ALT levels, reduced inflammatory infiltration and hepatocyte apoptosis in the liver. In these mice, serum levels of interleukin (IL)-10 was increased, while TNF- and IFN- were decreased as a result of VIP administration. Further investigation demonstrated the protective effects of VIP was IL-10-dependent and mainly in a cAMP-denpendent manner.

PP-003

Intrahepatic transplantation and tracing of hepatic oval cells in rats with fulminant hepatic failure

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Objective: To evaluate differences of Hepatic Oval Cells (HOC) labeled with green fluocescent protein(GFP) or CFDA-SE and further study therapeutic effect of HOC transplantation on rats with fulminant hepatic failure (FHF).

Methods: HOC obtained from proliferation model rats were labeled with GFP or CFDA-SE respectively. Cell fluorescence was observed under fluorescent microscope at 6, 24, 48 and 72h after labelling. CFDA-SE-labeled HOC (1×10^7cells/ml) was injected into livers of rats with FHF induced by D-galactosamine. The levels of Alb, ALT, AST, TBil in serum were measured at different time. The liver tissues were examined at day 3, 7, 14 and 21 after transplantation.

Results: The labelling proportion of HOC with GFP was 10% in contrast to 90% with CFDA-SE. No significant variations of cell viabilities were observed between two labelling approaches. The survival rate of HOC transplantation group was higher than that of control group, especially after 48 (9/15vs6/15) and 72 hours (9/15vs4/15). Decreased levels of ALT, AST, TBil and increased levels of Alb were achieved after HOC transplantation.

Conclusion: CFDA-SE was superior to GFP in labelling efficiency, yet the fluorescence intensity decreased progressively with cell division. HOC transplantation promoted the liver restoration from pathological damage, improved liver function and finally enhanced the survival rate of recipients. The fading and diffusion of fluorescence in liver tissues may suggest the proliferation and differentiation of transplanted-HOC.

PP-004

Sodium tanshinone IIA sulfonate protects mice from ConA-induced hepatitis via inhibiting NF-κB and IFN-γ/STAT1 pathways

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Sodium tanshinone IIA sulfonate (STS) is a water soluble derivative of tanshinone IIA, the main pharmacologically active component of *Salvia miltiorrhiza*. The aim of this study was to investigate the effect of STS on concanavalin A (ConA) -induced hepatitis (CIH) in mice, an experimental model of immune-mediated liver injury. C57BL/6 mice pretreated with STS released much less ALT into plasma in response to ConA challenge, and had reduced inflammatory infiltration and hepatocyte apoptosis in the liver. Thus STS protected mice from CIH. In STS-pretreated mice induced with CIH, we found abrogated TNF-α and IFN-γ production. Moreover, mRNA expressions of IFN-inducible protein-10 (IP-10) and macrophage inflammatory protein-1α (MIP-1α) in these mice were decreased. The mechanism of anti-inflammatory effects of STS may be attributed to its modulation of crucial inflammatory signaling pathways, including NF-κB and IFN-γ/STAT1. In conclusion, STS was capable of protecting mice from immune-mediated liver injury *in vivo* and the protection was associated with its suppressive effect on the production of important inflammatory mediators through modulating NF-kB and IFN-γ/STAT1 signaling pathways

PP-005

Quantitative Analysis of Acute Fatty Liver of Pregnancy by Multi-detector row CT (with Eighteen Cases Analysis)

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To investigate the quantitative criteria for diagnosing acute fatty liver of pregnancy (AFLP) on multi-detector row CT (MDCT). Among the 18 patients, there were 12 patients with mean CT number less than 50. Mean CT number as a criteria can conform AFLP, but it can be normal in some cases. The liver/spleen ratio (L/SP) was less than 1 in 9, and only one of which less than 0.7. L/SP is useful to diagnose fatty liver, but the modified vascular evaluation is more sensitive. In all, 3 appeared as normal findings of hepatic vessels (VE value = 0), 7 as obscure findings of hepatic vessels (VE value = 1) and 8 as hepatic vessel immersion (VE value = 2), there was no case which had an appearance of reverse or markedly reverse hepatic vessels display (VE value = 3 and 4). We selected VE value = 1 as a cutoff threshold, and there would be 15 patients conformed as AFLP. The interval from the date of initial onset symptom to the date of CT examination in 18 patients had good correlation with liver volume, L/SP, and VE value ($r = 0.52$, $r = 0.47$, $r = -0.49$, respectively; all P value < 0.05). VE value showed a significant correlation with L/SP and mean CT number, and VE value a moderate correlation with L/DA. The criteria of vascular evaluation value and liver volume is more reliable than that of CT number in quantitative diagnosis of acute fatty liver of pregnancy.

PP-006

Senescence marker protein 30 in acute liver failure: validation of a mass spectrometry proteomics assay

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Background: Our previous proteomic study showed that the senescence marker protein (SMP30) is selectively present in the plasma of a murine model of acute liver failure (ALF). The aim of this study was to validate this SMP30 expression in the plasma and liver tissues of mice and humans with ALF.

Methods: After the proteomic analysis of plasma from a murine model of D-galactosamine/lipopolysaccharide (GalN/LPS)-induced ALF by two-dimensional electrophoresis (2-DE) and mass spectrometry, the expression levels of SMP30 in the plasma and liver tissues were validated by western blot and RT–PCR analyses. These results were then confirmed in plasma samples from humans.

Results: These data validate the results of 2-DE, and western blot showed that SMP30 protein levels were only elevated in the plasma of ALF mice. Further analysis revealed that GalN/LPS induced the downregulation of SMP30 protein levels in liver tissues (by approximately 25% and 16% in the GalN/LPS-treated mice and in the treated mice that survived, respectively; $P < 0.01$). Hepatic SMP30 mRNA levels decreased by about 90% only in the mice that survived the GalN/LPS treatment. Importantly, plasma obtained from patients with ALF also contained higher levels of SMP30, about 3.65 ± 0.34 times those observed in healthy volunteers.
Conclusions: This study shows that SMP30 is not only a potential biomarker for the diagnosis and even prognosis of ALF. It also plays a very important role in a self-protective mechanism in survival and participates in the pathophysiological processes of ALF.

PP-007
Dynamic metabonomic analysis of BALB/C mice with different outcomes after D-Galactosamine/Lipopolysaccharide-Induced fulminant hepatic failure
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Background/Aims: Fulminant hepatic failure (FHF) is one of the most challenging gastrointestinal emergencies in clinical practice. Early identification of patients with FHF is very important. To construct a prediction model for early diagnosis and prognosis of FHF, we studied the dynamics of metabolic intermediates and metabolic profiles using a D-galactosamine/lipopolysaccharide (GalN/LPS)-treated BALB/c mouse model of FHF.
Methods: Levels of plasma metabolites were quantified using gas chromatography/time-of-flight mass spectrometry and data were processed using partial least squares discriminant analysis (PLS-DA).
Results: Distinct clustering differences were observed 5 h and 6 h after GalN/LPS treatment between mice that survived and those that died, but there were no differences between these groups at 4 h after treatment. At 5 h after treatment, plasma levels of some metabolites differed significantly between the survival, dead and control groups. Ketogenesis and the TCA cycle were inhibited in both the survival and dead groups, but in the dead group, the urea cycle was also inhibited and gluconeogenesis was elevated. PLS-DA indicated that principal component weighting was greatest for plasma levels of phosphate, β-hydroxybutyrate, urea, glucose and lactate. The Y-predicted scatter plot in the PLS model assigned samples to the survival or dead groups using an a priori cutoff of 0.10 with 100% sensitivity and specificity.
Conclusions: The PLS model based on metabonomics analysis can be used to predict outcomes well, and plasma levels of phosphate, ®-hydroxybutyrate, urea, glucose and lactate may constitute a set of markers for early diagnosis and prognosis of FHF.

PP-008
The beneficial effect of melatonin and pentofylline in hepatic ischemia and reperfusion injury in rats
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Total hepatic ischemia was induced for 35 minutes by Pingle occlusion. 128 rats were divided into four groups randomly. In group A, normal saline was used through caudal vein. In group B, MT was administered (10mg/kg, i.p.) at 75 and 30 min before ischemia, again just at the start of reperfusion, and after 60 and 120 min of reperfusion. In group C, PTX was given (50mg/kg, i.v.) 1h before laparotomy. In group D, PTX and MT were used together as above. Serum ALT, LDH and TNF-α were determined. Liver tissues were taken for determination of MDA levels, SOD levels, NO and ET-1. Expression of P-selectin and inductive NO synthase (iNOS) were evaluated immunohistochemistry. Seven-day survival rates were monitored.
At 2h, 4h of reperfusion , ALT, LDH and TNF-α in group B, C, D decreased compared with group A, and there were significant differences between group D and group B, C. At 4h of reperfusion, SOD of liver tissue in group D were significant lower than in group A. MDA and NO of liver tissue in B, D group were significantly decreased compared with group A, C. ET-1 activity were higher in group A than in group B, C, D. iNOS expression of group B, D were less severe than group A, C. Moreover, P-selectin staining was attenuated in A, C group compared with B, D group.
MT appeared to be significantly more potent than PTX in reversing the oxidative damage induced by I/R. PTX reduced reperfusion injury of the liver through significantly decreased secretion of cytokines. Our findings show that MT and PTX have beneficial effects against I/R injury and due to their synergistic effects, when administered in combination, may have a more pronounced protective effects on the liver.

PP-009
Prediction value of MELD scoring system on prognosis in patients with acute-on-chronic hepatitis B liver failure after plasma exchange and lamivudine treatment
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We use model for end-stage liver disease (MELD) scoring system to predict 3-month prognosis of patients with acute-on-chronic liver failure (ACLF) after plasma exchange (PE) and lamivudine treatment and study the predictive factors on the prognosis of patients. TBIL rebound rate of dead group was significantly higher than that of survival group (P<0.01). Univariate analysis showed that mortality was significantly related to age (P =0.003), treatment method (P =0.000), TBIL (P =0.010), MELD score (P =0.001), INR (P =0.014), pretreatment HBV DNA load (P=0.000), decline of HBV DNA load during therapy (P=0.013), encephalopathy (P =0.019) and hepatorenal syndrome (P=0.026). In multivariate analysis, in patients with MELD scores 30-40, treatment method (P=0.004), pretreatment HBV DNA load (P=0.008), decline of HBV DNA load during therapy (P=0.012), encephalopathy (P=0.018) and hepatorenal syndrome (P=0.033) were independent predictors of mortality; for MELD scores above 40, only MELD score (P=0.014) was independent predictive. PE significantly decreases the mortality of patients with MELD score 30-40. For ACLF patients with MELD score 30-40, a low viral load pre-treatment and quick decline of HBV DNA load are good predictors for the survival of PE and lamivudine treatment

PP-010
Evaluation of the incidence, maternal and neonatal complications of the HELLP syndrome and its risk factors
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Objective: The purpose of the present study is the evaluation of the incidence of the HELLP syndrome.
Method: A retrospective study was performed on 46628 deliveries during 5 years. There were 1811 cases(3.88%) of preeclampsia. 1380 cases of preeclampsia had complete documentation records which were evaluated .1196 patients were Iranian and 184 patients were Afghans. The preeclamptic patients were divided into the two groups; patients with HELLP syndrome (case group=164 cases) and patients without it. (control group= 1216 cases).
Results: From 1380 case of preeclamptic patients, 164 cases (11.9%) had HELLP syndrome. Who consisted 0.35% of total patients.
The mean platelet count were 81.3± 20.4 $\times 10^3$ /mm^3 and 198.1± 55.9$\times 10^3$ /mm^3 in the case and control group (p=000).
The mean proteinuria was higher in the case group (3.26± 0.9 gram vs2.53±1.25 gram (p<0.001).
The mean gestational age at the time of pregnancy termination were lower in the case group (33.85±4.21 and 35.9 ± 3.43 weeks) (p<0.002).
There were no significant difference between the two groups according to maternal age, parity, systolic and diastolic blood pressure and bilirubin and cesarean deliveries. Neonatal death [72 cases (44%) VS 170 cases (14%) (p=0.001)], Fetal complications [116 cases (71%) VS 389 cases (32%) (p=0.003)] maternal risk symptoms [116 cases (71%) VS 389 cases (32%) (p=0.002)]in the case group were higher than control group.
The incidence of HELLP syndrome was not different between Iranian (145 cases=12%) and Afghans, (19 cases=10%).
Conclusion: Race does not have impact on the incidence of HELLP syndrome

PP-011
Characteristics of acute liver failure patients in Selayang Hospital
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Objective: To determine the characteristics of acute liver failure patients in Selayang Hospital
Method: Records of seventy one consecutive patients admitted to the Selayang Hospital Hepatology Unit from September 2004 till May 2007 with hyperacute, acute and subacute liver failure were analyzed.
Results: *Gender* Forty five (63%) were females and 26 (37%) were

males.
Race Thirty six (51%) were malays, 27 (38%) Chinese and 8 (11%) Indians.
Age Ages ranged between 13-62 years with a median of 33.3 years. Thirty four(48%) were below thirty years old, 25(35%) between 31-50 years old and 12 (17%) were above 50 years old.
Jaundice to encephalopathy
Thirty six(51%) were hyperacute(1-7days), 25(35%) acute(8-28 days) and 10(14%) subacute(5-26 weeks).
Aetiology
Hepatitis B (acute or flare) – 11(15%)
Anti tuberculous drugs-10(14%)
Pregnancy related – 6(8%) fatty liver of pregnancy and 1(1%) HELLP syndrome
Dengue infection-6(8%)
Paracetamol overdose- 5(7%)
Traditional medication-5(7%)
Wilson's Disease- 4(6%)
Drugs apart from paracetamol and anti tuberculous-4(6%)
Autoimmune hepatitis – 2(3%)
Ischaemic hepatitis- 2(3%)
Budd Chiari –1(1%)
Amyloidosis –1(1%)
Hepatitis A-1(1%)
Unknown cause-13(18%)
Conclusion: Patients were mainly females and of a younger age group. The racial difference among the study population is similar to that of the general population. Majority of patients had hyperacute liver failure. Aetiology covered a wide range of diseases from the common to the extremely rare. Hepatitis B related acute liver failure and drugs, as expected, were the most common.

PP-012
Recombinant human hepatocyte growth factor for liver failure
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Aim: To evaluate the efficacy and safety of administration of recombinant human hepatocyte growth factor (rh-HGF) for liver failure.
Methods: The authors searched the literature to identify all available randomized controlled trials of rh-HGF plus comprehensive therapy (CT) with that of CT alone (Therapy I versus II) in treating liver failure by using the Cochrane Library, MEDLINE, EMBASE, CBMdisc, and CNKI. Meta-analysis was performed on the results of homogeneous studies.
Results: Twenty-one trials, all published in Chinese journals, involving 5902 patients were included. Meta-analysis showed that Therapy I, compared with therapy II, significant reduced the overall mortality (RR = 0.62; 95% CI, 0.59-0.66; p = 0.0001). Compared to other two subgroups (A and C), subgroup B had significant effect on mortality, RR and 95% CI were 0.76 [0.65, 0.89], 0.66 [0.60, 0.74], and 0.58 [0.53, 0.64], respectively. Besides, we found that a reduced in mortality favoring subgroup A+ compared to other two (subgroup B+ and C+) in patients with liver failure, RR and 95% CI were 0.34 [0.24, 0.49], 0.49 [0.44, 0.55], and 0.87 [0.82, 0.93] respectively. No serious adverse events were reported.
Conclusions: This review indicates Therapy I may reduce mortality in liver failure, especially in subacute liver failure and the early stage of liver failure. However, considering the strength of the evidence, additional randomized clinical trials are needed before Therapy I can be recommended routinely.

PP-013
Pediatric acute liver failure in treatment with albumin dialysis
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Background: Since 2004 Molecular Adsorbent Recycling System (MARS) was proposed as bridging procedure. The aim of our study is to assess its efficacy in children with PALF.
Material and Method: From 2004 we treated six pediatric patients with FHF: mean age 10,6 yr (3-15) , 4 famale and 2 male. In three cases the cause of FHF was unknown, in two cases induced by paracetamol overdose and one cause by Acute Hepatitis Virus –B related**.** Inclusion criteria: bilirubine > 15mg/dl, creatinine≥2 mg/dl, encephalopathy >II, INR >2.5. Continuous MARS treatment was carried on all patients with kit change every 10 hours.
Results: We observed a significant improvement of levels of bilirubine ($p<0.009$), ammonium ($p<0.005$), creatinine ($p<0.02$) , GCS ($p<0.002$) , PELD and predictive criteria as SOFA. Three children underwent to LT: 1 child died after five days for primary no function, 2 children are still alive after a median follow up of 14 months. In two children the MARS treatment has determined the resolution of clinical status without liver transplantation, one child dead before LT because of sepsi and Multi Organ Failure.
Conclusion: Liver support device MARS, can contribute to improve the clinical status in children with PALF awaiting liver transplantation

PP-014
Additive post-operative bilirubin and INR scores correlate with liver failure after major hepatectomy
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Background: The risk of liver failure (LF) after hepatectomy is a major concern. We aimed to identify specific post-operative blood parameters predictive of liver dysfunction after major hepatectomy which could serve as early warning indicator or outcome measure.
Methods: A retrospective cohort study was conducted from April 2007 to December 2007 to examine the relationship of combined stratified parameters including bilirubin, international normalized ratio (INR), alanine transferase (ALT) and albumin from post-operative days (POD) 0-6, and the occurrence of LF after major hepatectomy.
Results: Out of 35 patients with major hepatectomies, 11.4% developed LF with significantly longer hospital stay (LF:22-day vs. non-LF:11.5-day, P=0.005). Bilirubin and INR were significantly higher in those patients with LF across POD 2-6, whereas ALT and albumin did not differ between groups. Based on Child-Pugh score, threshold values of 50 μmol/L for bilirubin and 1.7 for INR were used to evaluate the impact of abnormal values on post-operative liver function. Bilirubin<50μmol/L was scored "0", otherwise "1"; INR<1.7 was scored "0", otherwise "1". The scores of these two blood parameters were summated from POD 0-6 to generate total bilirubin score (TBS) and total INR score (TIS). Both TBS and TIS ≧5 could be 75% predictive for LF, whereas both TBS and TIS <5 did not predict any patients with LF.
Conclusion: In patients with post-hepatectomy LF, both bilirubin and INR were consistently higher compared to those without LF, which is in agreement with previous report. This predictive equation will be verified in a prospective cohort of patients undergoing major hepatectomy.

PP-015
The effects of plasma from patients with acute on chronic liver failure on the proliferation and biotransformation function of C3A Cells
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Objective: To investigate the effects of plasma from patients with acute on chronic liver failure on the proliferation and biotransformation function of C3A cells in vitro.
Methods: C3A cells were incubated in 100% normal human plasma (NHP) and 100% abnormal plasma (AP) from patients with acute on chronic liver failure.
Results: The proliferation activities of C3A cells incubated in 100% AP for 24, 48, 72, 96 and 120 hours were significantly higher than that in 100% NHP. The diazepam metabolic amount of the C3A cells incubated in 100% AP for 24, 72 and 120 hours was lower than that in 100% NHP and was statistically different.
Conclusion: Compared with normal human plasma, the plasma from patients with acute on chronic liver failure has more obvious effect to facilitate the proliferation of C3A cells, but decreases partial biotransformation function of C3A cells.

PP-016
Effects of G-CSF on hepatocyte proliferation in rats with acute liver failure
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Objective: In the hope of providing a novel therapeutic approach for acute liver failure (ALF), we observed the influence of G-CSF on hepatocyte proliferation in rats with ALF.
Methods: One hundred and sixty of female SD rats were randomly divided into two groups: G-CSF therapy group and placebo control group. Acute liver failure models were induced by intraperitoneal injection of D-GalN 1.4g/kg into the rats after 12 hours fasting. Another six rats without any treatment were taken as normal control.

After 2 hours of D-GalN inducing, the animals in G-CSF therapy group were injected hypodermically with recombinant human G-CSF (rhG-CSF) 50μg/kg·day for 5 days, while the animals in placebo control group were injected by saline as placebo. Blood and liver samples were collected from 6 rats of each group at the time of 1, 3, 5 and 7 days after D-GalN inducing.

Results: The survival rate in G-CSF therapy group markedly improved (53.3% vs 33.3%, P=0.027). The serum levels of alamine aminotrangerases (ALT) in the animals were increased remarkably. The Ki-67 labeling index (Ki-67-LI) in G-CSF therapy group was higher than that in placebo control group on the third day and the seventh day after D-GalN induction (28.03% vs 22.62%, P=0.04 and 32.22% vs 28.97%, P=0.016, respectively).

Conclusion: The administration of G-CSF can significantly increase the survival rate, improve liver function and accelerate the hepatocyte proliferation in rats with ALF induced by D-GalN.

Poster Session – Alcoholic Liver Disease

PP-017

The up-regulated hepatic expression of TLR4 mRNA in a nonalcoholic steatohepatitis rat model induced by high-fat diet

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Aim: Nonalcoholic steatohepatitis (NASH) animals are more sensitive to LPS hepatic injury. Therefore, the hepatic expression of LPS receptor TLR4 in a rat NASH model was observed. Methods: Male SD rats were fed a high-fat diet (HF group). These rats were killed at week 4, week 8, week 12, week 16, and week 24. Hepatic expressions of CD14 were observed by immunohistochemistry and TLR4 were detected by RT-PCR. Levels of serum endotoxin were detected.

Results: At the 8th week of "fatty liver" period, the hepatic expressions of CD14（25.9±11.9）and TLR4mRNA（1.75±0.81）were up-regulated in HF group compared to the control group（12.4±5.7 and 0.98±0.33）. Hepatic expressions of two receptors increased with the occurrence of "steatohepatitis" at the 12th week（61.8±21.9 and 1.88±0.72）, reached the maximum at 16th week（71.5±21.3 and 5.64±0.87）, and decreased slightly at the 24th week （67.7±16.6 and 4.98±0.72）. Serum endotoxin levels didn't increased significantly until the 24th week (0.229±0.06 EU/L *vs* 0.152±0.03 EU/L,).

Conclusion: The hepatic expressions of TLR4 mRNA were up-regulated in the process of inducing a rat NASH model by high-fat diet, but the serum endotoxin levels didn't increased at the same time (except the latest period). The up-regulated hepatic expression of TLR4 mRNA may be related to the increasing sensitivity to LPS injury of NASH animals.

PP-018

Reduced expression of the nuclear receptor FXR in non-alcoholic fatty liver disease accompanied with cholesterol gall stone

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Background: Some epidemic researches suggested prevalence of cholesterol gall stone (GD) is higher in non-alcoholic fatty liver disease (NAFLD) than in general population. These two diseases were probably related with the derangement in metabolism of bile aid and lipid.The aim of the present study was to analyze the hepatic expression of some nuclear receptors involved in bile acid and lipid metabolism in human NAFLD accompanied with GD.

Methods: 50 patients were diagnosed with imaging detection and divided into three groups: NAFLD accompanied with GD（20）、simple GD（20） and normal control（10）.Surgical liver biopsies were obtained from these subjects. The mRNA and protein expression of FXR and its target or related genes were detected by RT-PCR and immunohistochemistry.

Results: As compared with normal control,mRNA expression of FXR、BSEP and PGC-1α was decreased significantly in the group of NAFLD accompanied with GD, SREBP-1c and HMGCoA reductase was increased significantly;mRNA expression of PGC-1αand BESP was decreased significantly and HMGCoA reductase was increased significantly in the group of simple GD.No differences were detected the expression of CYP7A1,which is the key enzyme involved in bile acid synthesis. Immunohistochemistry stain show that the protein expression of FXR was decreased significantly and SREBP-1c was increased significantly in the group of NAFLD accompanied with GD.

Conclusions: FXR and its related gene appears to play a role in the pathogenesis of NAFLD accompanied with GD in humans.FXR may be the potential therapy target on the control of metabolic syndrome.

PP-019

The mechanisms and significance of Q-Tc interval prolongation and Q-T dispersion changgenation in hepatic cirrhosis

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To investigate the mechanisms and significance of Q-Tc interval prolongation and Q-T dispersion changgenation in hepatic cirrhosis.Eighty six cases of liver cirrhosis and thirty control subjects were studied.Rated corrected Q-Tc and QTd, the liver function tests of cirrhosis patients ,included PTA 、ALb 、TBIL, evaluated ascetic fluid and hepatic encephalopathy. correlation coefficiencies were derived by linear regression analysis .Variables were correlated with Q-Tc by multiple regression analysis.Q-Tc in cirrhosis patients prolonged in 36 patients and 1 controls, 41.9% vs 3.3%,P<0.01.Q-Td was (48.7±18.6)ms and (34.6±11.1)ms in controls ,P<0.01.Multiple regression analysis showed Child-Puch score,TBIL, ascetic fluid was independently correlated with Q-Tc interval. Q-Tc interval prolongation and Q-T dispersion changgenation are co-effected by multiple factors.Since Q-Tc interval prolongation and Q-T dispersion scale up are the common motivation of ventricular arrhythmia,to avoid drugs those will influence cordis-repolarization is necessary when the patients being serious complications or will be on major operations.

PP-020

Effect of tea polyphenols on expression of nuclear factor κappa B and cyclooxygenase2 in rats with alcoholic liver diseases

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Objective: To investigate the effect of tea polyphenols on expression of nuclear factor κappa B and cyclooxygenase2 in rats with ALD.

Methods: 32 female Wistar rats were randomly divided into three groups: control group treated with corn oil,model group with alcohol and corn oil,TP group with corn oil and alcohol plus TP.All treatments were injected into stomach through intragastric tubes.Liver samples were analyzed for histopathology with light microscope and transmission electron microscope,and the expression of NF-κB and COX-2 with RT-PCR and immunohistochemistry.Levels of ALT、AST and MDA in serum were measured.

Results: The level of ALT in serum increased significantly in the model group compared with those in the control group.To compare with the model group,the levels of ALT、AST and MDA decreased significantly. NF-κB and COX-2 expression in the liver by RT-PCR and immunohistochemistry increased significantly in the model group, compared with the control group and the TP group.

Conclusion: TP can diminish alcohol-induced liver injury in rats through inhibiting NF-κB activation and decreasing COX-2 level.

PP-021

Protective effects of cichorium indybus and tinospora cordifolia in alcohol induced liver damage

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Aim: The present study was designed with the aim to determine the hepato protective and curative actions of cichorium indibus and tinospora cordifolia in alcohol induced liver damage in rats.

Materials and Methods: Male albino rats were divided into 3 groups viz controls (groupI), alcohol induced liver damage (group II) and cichorium and tinospora treated (group III). Liver damage was produced by administering 18% ethanol/ day /100 gms body weight of male albino rats orally for a period of 90 days. In the present study aqueous extract of cichorium indibus and tinospora cordifolia were administered orally from 61st day to 90th day to alcohol induced liver damage rats.Blood samples were collected for estimating liver glyco proteins, serum hydroxy proline, hexose, hexosamine, sialic acid levels in experimental groups and compared to controls.

Results: The level of hexose, hexosamine and sialic acid are significantly decreased in alcohol induced rats when compared to controls. The decreased levels of the above parameters are normalised on treatment with cichorium indibus and tinospora cordifolia. The increased level of hydroxyproline in alcohol induced rats was normalized on cichorium indibus and tinospora cordifolia treatment.

Conclusion: The results of our study indicate the protective and curative effects of the cichorium indibus and tinospora cordifolia in alcohol induced liver damage.

PP-022

Epidemiological survey of fatty liver disease in the Northwest, China

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Objective: To evaluate the characteristics of ethanol consumption, the incidence of alcoholic and non-alcoholic fatty liver disease in a general population of Northwest, China. And to analyze their related risk factors.

Methods: A cross-sectional survey with multiple-stage stratified cluster and random sampling was performed. All samples aged 18 and above were gotten in different occupation crowds and both city and

rural people of Northwest, China. Questionaire, height, body weight, waist circumference, biochemical tests and ultrasonographic examination of liver were undertaken.
Results: A total of 2300 subjects took part in the survey. Of which 1726(75.0%) were males and 574 (25.0%) were females. The prevalence of drinking, alcoholic and non-alcoholic liver disease were 66.2%, 8.7% and 14.7%, respectively. Multiple logistic regression demonstrated that alcoholic liver disease was closely related to age, sex and average daily alcohol intake. Non-alcoholic fatty liver disease was positively correlated to age, female, hyperlipoidemia, diabetes mellitus and BMI.The prevalence of obesity of multiple metabolic disorders was the highest in the fatty liver .The trend test show that the prevalence of fatty liver were increased by the increased number of diagnostic criteria for the metabolic syndrome. **Conclusions:** The drinking-rate is higher in the Northwest, China. Alcoholic and non-alcoholic fatty liver disease in the Northwest is highly prevalent and non-alcoholic fatty liver disease is the major type. Drinking, hyperlipoidemia, diabetes mellitus and obesity are the major risk factors. Fatty liver is closely related to multiple metabolic disorders.

PP-023
Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with alcoholic liver disease (ALD)
Surapaneni Krishna Mohan[1], Vishnu Priya V[2]
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Background: The exact pro-oxidant and antioxidant status in Alcoholic Liver Disease (ALD) patients is still not clear. This work was undertaken to assess oxidative stress and anti oxidant status in patients with ALD.
Methods & Materials: Erythrocyte GSH was measured by the method of Beutler et al. Ascorbic acid levels were measured by the method of Tietz. Plasma vitamin E levels were measured by the method of Baker H et al. MDA was determined as the measure of thio barbituric acid reactive substances (TBARS). SOD activity in the hemolysate was measured by the method of Misra & Fridovich. Activity of catalase was measured by the method of Beers and Sizer. GP_X activity was measured as described by Paglia and Valentine in erythrocytes and Plasma GST activity was measured as described by Warholm et al. The study group comprised of forty-five male patients of alcoholic liver disease, having history of alcohol intake for more than five years with daily intake of 80-160 gm continuously. Patients were subjected to detailed clinical examination and laboratory investigations. An Equal number of normal age & sex matched healthy individuals with similar socio-economic status were selected as controls.
Results: It was observed that there was a significant increase in erythrocyte MDA levels, SOD, GP_X & plasma GST activities and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with alcoholic liver disease when compared to controls.
Conclusions: The results of our study suggests higher oxygen free radical production, evidenced by increased MDA and decreased GSH, ascorbic acid, vitamin E and Catalase activity, support to the oxidative stress in ALD. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

PP-024
The protective effect elicited by ethanol precondition on the liver from ischemia-reperfusion injury in rat
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Southwest Hospital & Institute of Hepatobiliary Surgery, Third Military Medical University, Gaotanyan Mainstreet, Shapingba District, Chongqing, P.R.China.
Background: This study was to investigate the effect of the ethanol precondition on the ischemic rat liver.
Methods: Adult male Wistar rats were pretreated with the 40% ethanol of 5g /kg.bw and rationally divided into different group: normal control group (N), only ethanol group (E), only ischemia-reperfusion group (IR), Natural Solution pretreated group (NPC), ethanol precondition group (EPC) and ischemic precondition group (IPC). The portal vein blood blocked procedure was adopted for the liver ischemia model and the ischemic duration was set to 90,120,130 min respectively. The mortality of the animal, serum ALT /AST activities, hepatocyte apoptosis and histological observation of the liver were included. Hepatic Hsp70 expression was analyzed by Western blot.
Results: A good histologic construction of the liver speciman and less elevation of ALT/AST activities with enhanced expression of Hsp 70 could be found in the EPC group, also with less apoptosis compared with other groups.
Conclusions: Enhanced bearance from the I/R injuries of the rat liver can be induced by ethanol precondition, which may be a novel measurement to fight against the liver I/R injury in future.

Poster Session – Fibrosis

PP-025

Transdifferentiation of hepatocytes after bile duck ligation rats

Bingfeng Qiu[1], Ping Liu[2,3], Jinxing Dou[1], Cheng Liu[1], Mingyu Sun[1], Dingzhu Shen[1], Chenghai Liu[1], Yiyang Hu[1], Lieming Xu[2,1]

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Objective: To investigate the transdifferentiation of hepatocytes after bile duck ligation rats.Methods: Rats were randomly assigned to three groups: 1) sham control; 2) BDL. Rats were sacrificed at 1,2,3,4,5 weeks and the liver were weighed. The protein expression of CK 7, α-SMA, c-kit were measured by Western blot. Hepatic histology were observed by HE and Fouchet,s stainings. Double immunofluorescences of CK 7 and HepPar/α-SMA/ c-kit, Albuin and α-SMA , HepPar and c-kit were performed on frozen sections.Results: Hepatic weight increased significantly, the expression of CK 7 and α-SMA increased dramatically while c-kit was unchanged in 5 weeks. Bile duct like epithelium cells comprising cholochrome were founded at 5th weeks. Furthermore, not the colocalizations of CK-7 and HepRar/α-SMA, but that of Albuin and α-SMA were observed. Conclusions: Hepatocytes could transdifferentiate toward bile duct epithelium cells in cholestasis in vivo, and the cells transdifferentiate into myolfibroblast cells later.

Figure 1

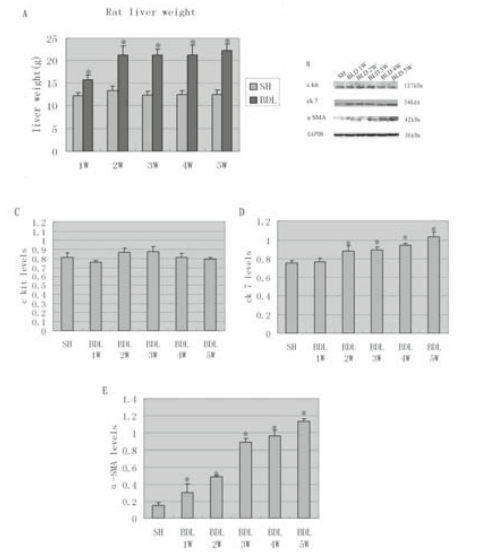
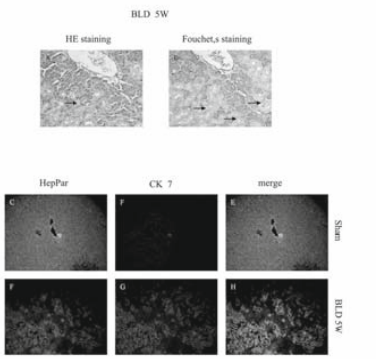


Figure 2



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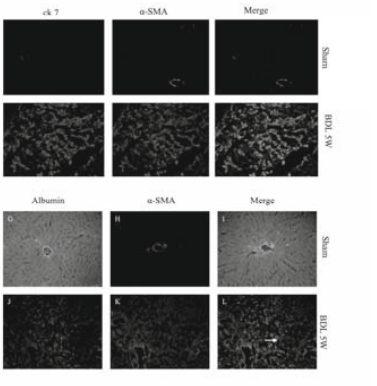
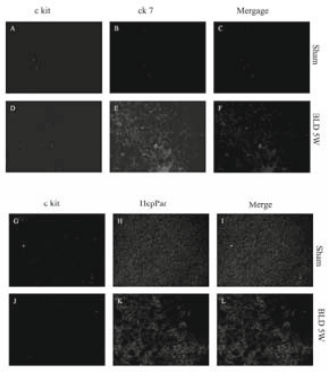


Figure 4



PP-026

Angitensin converting enzyme 2 is a negative regulator of liver fibrosis

Qian Huang[1], Qing Xie[1], Hui Wang[1], Cuicui Shi[1], Lanyi Lin[1], Hong Yu[1]

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Background: The rennin angiotensin system (RAS) plays a major role in liver fibrosis. A new homologue of Angiotensin converting enzyme –ACE2 has been identified to be negative regulator of the RAS by degrading ang II to ang1-7.The role and expression of ACE2 remains elusive in liver fibrosis.

Aims: To investigate the role and expression of ACE2 in liver fibrosis in vivo and examin the effect of losartan on ACE2 in liver fibrosis.

Methods: Male SD rats were random divided into CCL4 group, losartan treated group and control group. CCL4 group and losartan treated group received injection of CCL4 for up to 6 week and were sacrificed and analyzed at various time points. Liver pathology was analyzed by HE and masson staining, and real-time PCR for ace2, ace, TIMP-1.

Result: Iimmunohistochemistry demonstrated ACE2 protein were expressed in cell membrane and cytolymph.Only low levels of ACE2 protein were detected in control rat liver tissure but these were increased in CCL4 group, losartan treated group. Resltime-pcr analysis revealed ACE2 mRNA levels were significantly upregulated after 2 weeks of of injecting CCL4. ACE2 mRNA levels were significantly higher about 2 fold,17 fold,11 fold in losartan treated group compared to control gruop at various time points ($p<0.01$)。 Losartan treated group could significantly increase ACE2 mRNA levels compared to CCL4 group. CCL4 group displayed increase of TIMP-1 and ACE mRNA levels, but losartan treated group significantly inhibited their increase ($p<0.01$).

Conclusion: The RAS is activated following CCL4 injection. ACE2 is upregulated in liver fibrosis. ACE2 is negative regulator of liver fibrosis. Upregulating ACE2 expression may improve liver fibrosis.

PP-027

Remodeling of liver fibrosis accompanying enhanced v 3 integrin receptor-mediated ligand binding in rats

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Objective: To investigate expression of αVβ3 integrin and its ligand binding capability in both hepatic stellate cells (HSC) and fibrotic tissue.

Methods: Expression of αVβ3 integrin was investigated by real-time PCR, Western blot, and fluorescencet stained respectively. Labelled αVβ3 integrin ligand of cRGDfK and cRGDyK with carbofluorescenc (FAM) and iodine-125 radioactive isotope as probes. Incubated HSCs with the probes, and internalization of probes was analyzed using fluorescence tracing and flow cytometry. The equilibrium dissociationconstant (Kd) and maximal binding capacity (Bmax) for HSCs were calculated by radioligand binding assay (RBA). Ligand binding of tissues was observed by tissue autoradiography.

Results: The αvβ3 integrin exclusively located on activated HSCs. Expression of αvβ3 increased in fibrotic liver tissue compared with control ($P<0.05$), and co-located with α-SMA. After incubated with 10 μM probes for 45 minutes the activated HSCs increased their fluorescence intensity by 6.6-fold as compared to background, and the intensity enhanced in a concentration- and time-dependent manner. Kd was 4.81×10^{-9} mol/L and Bmax per cell was 1.27×10^{4} nearly. Autoradiography shown liver tissue radioactivity enhanced significantly in liver fibrosis rats ($P<0.05$).

Conclusions: The expression of integrin αVβ3 was up-regulated following liver architecture remodeling. Fibrotic liver enhance uptake of cRGD ligand by a receptor-mediated pathway. This provides a promising strategy for developing targeted therapeutic or diagnostic agents in chronic liver disease.

PP-028

Sphingosine 1-phosphate (S1P) mediated homing of bone marrow mesenchymal stem cells to the fibrotic liver

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Background & Aims: Hepatic myofibroblasts play a key role in liver fibrotic process. Myofibroblasts of bone marrow origin have recently been highlighted in fibrotic liver. However, little is known about the mechanisms that control their in vivo mobilization. In the present study, we confirmed that bone marrow mesenchymal stem cells (MSCs) can migrate to the injured liver and differentiated into myofibroblasts, and investigated the molecular mechanisms of S1P-mediated homing of MSCs to the fibrotic liver.

Methods: ICR mice were lethally irradiated and received bone marrow transplants, among of which MSCs were prepared from Green Fluorescent Protein transgene mice. Carbon tetrachloride (CCl_4) was used to induce liver fibrosis. MSCs-derived myofibroblasts were tracked in liver sections by immunohistochemistry.

Results: MSCs contributed significantly to myofibroblast populations in the fibrotic liver. Specifically, S1P levels in the liver tissues and serum of CCl_4-treated mice were increased through up-regulation of sphingosine kinase, down-regulation of S1P phosphatase and un-change of S1P lyase, which shown that S1P was involved in the development of liver fibrogenesis. Moreover, S1P induced the migration of MSCs in vitro, shown that S1P-induced MSCs migration acted through S1P3 receptor in a pertussis toxin-sensitive manner, suggesting that S1P3-initiated signaling was mediated by the G αi-protein coupled pathway. Furthermore, S1P-mediated migration of MSCs repaired the activities of ERK, the small GTPase Rho and Rac.

Conclusions: Our results demonstrate that S1P-mediated homing of MSCs to the fibrotic liver. It is promising to design a new strategy for the treatment of liver fibrosis based on the signaling pathways underlying S1P-mediated MSCs mobilization.

PP-029

Study of peroxisome proliferator-activated receptor gamma targeted agonist rosiglitazone prevents hepatic fibrosis associated with non-alcoholic steatohepatitis in mice

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Objective: Our objective was to clarify the protective effect of peroxisome proliferator-activated receptor gamma (PPARγ) agonist, rosiglitazone, on advanced hepatic fibrosis induced by feeding mice with high fat, choline-methionine deficient diet through modulating expression of transforming growth factor beta 1 (TGFβ1) and connective tissue growth factor (CTGF).

Methods: An experimental model of non-alcoholic steatohepatitis/hepatic fibrosis was established by feeding male C57BL6/J mice with high fat, choline-methionine deficient diet (MCD) for eight weeks. Control mice were fed choline-methionine supplemented diet, and treatment animals were given MCD diet plus rosiglitazone (50mg/kg/day) for 8 weeks. Hepatic steatosis, inflammation and fibrosis were graded and staged under H&E and Masson staining. Expression of mRNA and protein of TGFβ1 and CTGF was analyzed by RT-PCR and immunohistochemistry, respectively.

Results: Hepatic histopathology were normal in control mice. Severe steatohepatitis with inflammatory infiltration, hepatic spot or focal necrosis, severe portal and sinus hepaticus fibrosis were developed in mice fed with MCD diet, and compared to control animals, mRNA and protein expression of TGFβ1 and CTGF were notedly increased, (*P value*<0.05 and 0.01 respectively). Rosiglitazone supplement prevented steatohepatitis /hepatic fibrosis progression, in consistent with the liver histology, both mRNA and protein expression of TGFβ1 and CTGF were significantly down regulated by rosiglitazone (both *P value*<0.05).

Conclusions: MCD diet can induce hepatocytes damage and liver fibrosis, which might be inhibited by down-regulation of expression of TGFβ1 and CTGF with PPARγ targeted agonist, rosiglitazone.

PP-030

Preparation and anti-fibrotic effects of solid lipid nanoparticles

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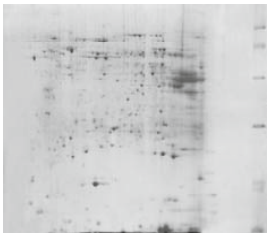
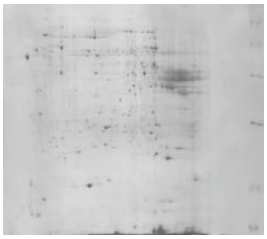
Objective: To prepare solid lipid nanoparticles (SLN) loaded with silibinin (SIL) extracted from traditional Chinese medicine and study the anti-fibrotic effects in model rats of liver cirrhosis.

Methods: SIL-SLN was prepared by high pressure homogenization. Experimental rats were randomly divided into normal control group, model control group, SIL- suspension oral group, SIL-SLN oral group, and SIL-SLN intravenous (iv) group. The anti-fibrotic effects of normal drug delivery and SLN of SIL were compared. Results The SIL-SLN prepared by high pressure homogenization was spherical and regular in shape. The particle diameter was (157±8) nm and the zeta potentials was (−35.36±2.68)mv. The mean entrapment efficiency was 95.64% and the mean drug content was 1.501 $mg \cdot mL^{-1}$. After administration of SIL for 8 weeks, the gray values of Masson's trichrome staining in rats liver tissues of SIL-SLN oral group and SIL-SLN iv group were 4.73±1.35 and 2.26±0.42 respectively, and the differences were significant comparing with SIL-suspension oral group (7.26±1.72, $P<0.05$). The anti-fibrotic effect of SIL-SLN iv group was better than SIL-SLN oral group ($P<0.05$).
Conclusions: The anti-fibrotic effect of SIL-SLN was much higher than SIL-suspension, especially by intravenous injection. SLN is a new targeting drug delivery of traditional Chinese medicines.

PP-031
The distribution and significance of insulin-like growth factor binding protein2，6 and 7（rP1） in patients with liver fibrosis
Lixin Liu[1,2], Xiao-xia Tian[2], Gui-xiu Qin[1], De-wu Han[2]
[1] *The First Hospital of Shanxi Medical University,* [2] *Institute of Liver Diseases of Shanxi Medical University*
Objective: To study the distribution and significance of IGFBP2,6 and7（rP1） on human with liver fibrosis or cirrhosis. Meanwhile to investigate relationship between the expression of IGFBP2, 6, 7（rP1） and the contents of collagen fiber , TGFβ1, TGFRI and CD14 in hepatic tissue.
Methods: 42 patients were randomly divided into 3 groups, including 18 cases of hepatic fibrosis, 18 cases of liver cirrhosis and 6 control cases of hemangiomas of liver surrounding healthy tissue. H&E, VG and immunohistochemical staining of TGFβ1, TGFRI, IGFBP2, 6, 7(rp1) and CD14 were perfomed.
Results: The contents of IGFBP2, IGFBP6, IGFBP7(rp1) were significantly increased in both hepatic fibrosis and cirrhosis group as compared with control group. And the contents of IGFBP2, IGFBP6, IGFBP7(rp1) were significantly increased in cirrhosis group as compared with hepatic fibrosis group. Also the contents of TGFβ1、TGFRI、CD14 were significantly increased in hepatic fibrosis and cirrhosis group as compared with control group. And the contents of TGF-β1、TGFRI、CD14 were significantly increased in cirrhosis group as compared with hepatic fibrosis group. While there were the positive correlation between the expression of IGFBP2, 6, 7（rP1） and the expression of collagen fiber , TGFβ1, TGFRI and CD14 in hepatic tissue.
Conclusions: IGFBP2, IGFBP6, and IGFBP 7（rP1） are closely correlated with collagen fiber , TGFβ1, TGFRI and CD14. They may influence the progression of liver fibrosis.

PP-032
Differential analysis of associated- proteome in hepatic fibrosis tissue
Luo Xinhua[1]
[1] *Department of Infectious Diseases, Affiliated Hospital of Guiyang Medical College*
Objective: To develop the 2-DE profiles of proteome from hepatic fibrosis tissue and preliminary analyze the differential expression of proteome.
Methods: The differential expression of proteins were analyzed by imagine analysis and MALDI-TOF-MS after the total protein was extracted from the hepatic fibrosis tissue and the normal liver tissue by 2-DE. 3 proteins were verified by in Western-blot.
Results: Fifty-nine differential expressed-protein were found in the proteome profiles analysis of these two types of tissue, among which 30 protein spots were up- regulated and 29 protein spots were down-regulated in hepatic fibrosis tissue. Western-blot results of 14-3-3β、DJ-1 and PEBP were consistent with those by the 2-DE examination.
Conclusions: Some differential expressed-protein were found in the hepatic fibrosis tissue and the normal liver tissue.

PP-033
Mechanism of hepatic stellate cells migration during liver fibrosis
Li Yang[1], Chang-qing Yang[1], Wen-zhuo Yang[1]
[1] *Tongji hospital of tongji university*
Background & Aims: To study the mechanism of migration of hepatic stellate cells (HSC) within the space of Disse microenvironment during liver fibrosis, and to explore the novel pathogenesis of liver fibrosis from the view of cell migration.
Methods: A modified in vitro Boyden chamber system was used to partially mimic microenvironment of Disse space of normal or fibrosis. The mechanism of migration of HSC was studied by cell migration assay, zymography and immunoblotting.
Results: Stimulation of HSCs with platelet-derived growth factor (PDGF)-BB, transforming growth factor (TGF)-β1, and/or epithelial growth factor (EGF) resulted in an increase in their migratory capacity and up-regulated matrix metalloproteinase (MMP)-2 activity. Additionally, we show that type I collagen by itself induced migration of HSC, while type IV collagen inhibited it. Migration induced by PDGF-BB, TGF-β1, and collagen I could be inhibited by α1- and/or α2-integrin blocking antibodies, collectively suggesting an integrin-dependent, MMP-2–mediated migration of HSC during liver fibrosis.
Conclusions: In liver fibrosis, alterations within the space of Disse microenvironment facilitate migration of HSC, the mechanism is associated with up-regulating of MMP-2 and with mediation of α1β1 and α2β1 integrins. Extracellular matrix (ECM) by it self showed feedback actions to migration of HSC.

PP-034
CGX protects against hepatofibrosis via antioxidative actions in animal model
Jing-Hua Wang [1], Jang-Woo Shin[1], Jin-Young Son[1], Chong-Kwan Cho[1], Chang-Gue Son[1]
[1] *Daejeon University*
The cirrhotic change of liver tissue is a critical step that impacts liver function and clinical outcome. The development of antifibrotic agents has been a major focus for the treatment of cirrhosis. To investigate the antifibrotic effects and associated mechanisms of CGX, a hepatotherapeutic drug composed of thirteen herbs, rat liver fibrosis was induced by DMN (10 $mg\ mL^{-1}\ kg^{-1}$ ip on 3 consecutive days per week for 4 weeks) and the animals were treated with CGX. Histological examination showed that CGX treatment clearly ameliorated the increased levels of collagen and hydroxyproline in the diseased liver tissues, as well as the serum biochemical parameters of albumin, AST, ALT, ALP, and bilirubin. Antioxidant proteins, GSH content, catalase, and superoxide dismutase activities were also maintained in the CGX-treated groups. In addition, expression profiling of antioxidant- and antifibrosis-related genes by RT-PCR revealed major contributions by *iNOS, TGF-β, TNF-α*, and *PDGF-β*, but not by *TIMP-1, TIMP-2, MMP-2*. These results add to the therapeutic evidence for the clinical efficacy of CGX in chronic liver disorders and for the antioxidative properties that underlie its antifibrotic effects.

PP-035
Hepatic gelatinase activity expression in situ during hepatic fibrosis in rat
Qian Chen[1], Hongyan Cui[2], Yanyan Tao[1], Chenghai Liu[1,3]
[1] *Liver Disease Institute, Shanghai University of Traditional Chinese Medicine，Shuguang Hospital,* [2] *Liver Disease Institute, Shanghai University of Traditional Chinese Medicine，Shuguang Hospital,* [3] *E-institute of TCM Internal Medicine, Shanghai Municipal Education Commission*
Aim: Observe Col-IV expression and gelatinase activity dynamically during experimental liver fibrosis formation and regression, to understand gelatinase and Col-IV role in fibrogenesis.

Methods: The liver fibrosis model was induced by DMN in rats. The models were observed at 1d, 3d, 1w, 2w, 3w, 4w after initial DMN intoxication, and 5w, 6w, 7w, 8w actually at 1-4w after stopping DMN.
Results: Col-IV, Col-I protein expressions and gelatinase activity gradually increased as liver fibrosis developed after DMN injection, and decreased after DMN stopping. Col-IV and gelatinase mainly expressed at hepatic sinusoidal area and fibrous septa, some at hepatocytes in model rats, whose expression levels increased as fibrosis formed, but decreased as fibrosis regressed.
Conclusions: Gelatinase activity and Col-IV protein increased at the same location during liver fibrogenesis, indicating that accumulation and degradation of Col-IV happened simultaneously, while gelatinase play a pivotal role in degrading Col-IV, destroying liver microenvironment and liver remodeling.

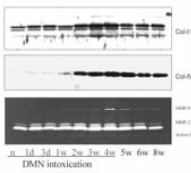


Fig 1. Col-IV/I proteins and gelatinase activity expression during liver fibrosis formation and regression.

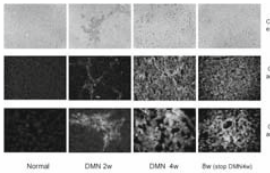


Fig 2. Col-IV proteins and gelatinase activity *in situ* expression during liver fibrosis formation and regression.

PP-036
Effect of FuzhengHuayu decoction on hepatocytic apoptosis in mice induced by LPS
Chenghai Liu[1], Tao Zhou[1], Yanyan Tao[1]
[1] *Shanghai University of Traditional Chinese Medicine, Institute of Liver Diseases*
Objective: To explore effect of FuzhengHuayu (FZHY) decoction on hepatocytic apoptosis and necrosis on acute hepatotoxin due to LPS in Mice.
Method: Mice were designed into normal, model and FZHY group. Mice of FZHY group took Fuzhenghuayu decoction twice a day for 3days. One hour after being feed at the fourth day, mice of model and FZHY groups were intraperitoneally injected with LPS and GalN. All mice were sacrificed 6 hours after injection. The serum ALT and AST were assayed. Hepatocyte apoptosis and necrosis were checked with TUNEL and HE staining. The protein expressions of bcl-2/bax and Caspase3 were analyzed with Western blotting.
Result: Serum ALT and AST activity were increased in model group, and FZHY decoction decreased the increased ALT and AST. On morphology in general, the liver in model group were overly swollen, congested, and bleeding district. HE staining showed many infiltrated inflamminatory cytes and necrotic hepatocytes in liver in model group, but FZHY decreased infiltrated inflamminatory cytes and necrotic hepatocytes. TUNEL staining show no apoptotic hepatocytes in normal liver, many apoptotic hepatocytes were dispersed in liver in mice of model group. After treatment with Fuzhenghuayu decoction, Hepatocytic apoptosis index in the mice of treatment group was decreased. The gene and protein expression of bcl-2 was significantly up-regulated in liver in treatment group, but those of bax and Caspase3 were down-regulated.
Conclusion: Fuzhenghuayu decoction decreased hepatocytic apoptosis and necrosis in acute hepatotoxin due to LPS in mice, and would be used in acute and chronic liver diseases.

PP-037
Assessment of hepatic fibrosis in chronic liver disease - Usefulness of the strain rate
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[1] *Masuko Memorial Hospital,* [2] *Toshiba Medical*
Purpose: The development of hepatic fibrosis in patients with chronic liver disease increases the risk of liver cancer. Assessing the degree of hepatic fibrosis is therefore one of the most important factors in therapeutic planning. Strain Rate Imaging is a new method based on Tissue Doppler Imaging (TDI). This method is useful for eliminating the effects of translational motion or tethering of the myocardium and for evaluating the local contraction and expansion of the myocardium. This time, it mede comparative study with Fibroscan in 11 cases.
Methods: Strain Rate Imaging was performed using a diagnostic ultrasound system (Aplio™, Toshiba Medical Systems Corporation, Tochigi, Japan) in a total of 47 subjects: 25 in the chronic hepatitis group, 12 in the cirrhosis group, and 10 in the normal control group. Dynamic images were acquired as raw data in Harmonic TDI mode.
Results: The mean strain value was 0.156 in the chronic hepatitis group, 0.055 in the cirrhosis group, and 0.26 in the normal control group.The correlation was not thought to be Fibroscan.
Conclusion: The results of the present study suggest that this noninvasive method permits quantitative assessment of the degree of hepatic fibrosis to be performed easily and in a short time. It is expected that the accuracy of the Strain Rate Imaging method in determining the degree of hepatic fibrosis will be improved when it is used in combination with histological examination.

PP-038
Effects of salvianolic acid B on hepatocytic apoptosis in vivo and in vitro
Xiuchuan Yan[1], Yanyan Tao[1], Li Shen[1], Chenghai Liu[1,2]
[1] *Liver Disease Institute, Shanghai University of Traditional Chinese Medicine，Shuguang Hospital，Shanghai 201203,* [2] *E-institute of TCM Internal Medicine, Shanghai Municipal Education Commission, Shanghai, 201203*
Objective: To study the effect of Salvianolic acid B (SA-B) on hepatocytic apoptosis *in vivo* and *in vitro*.
Methods: The model was induced by ip of LPS and GalN for 6h in mice, and treated with 10mg/Kg SA-B just before intoxication. SMMC7721 (hepatocyte cell line) apoptosis was stimulated with Act D/TNFα for 6h and incubated with 1μM and 10μM SA-B. Serum ALT and AST activity were measured. Hepatocytic apoptosis in tissue was detected with TUNEL assay. Apoptosis in cell line was determined by DNA electrophoresis, flow cytometry and high content screening machine (Kinetic Scan).
Results: SA-B decreased the ALT/AST levels in model rats, improved hepatic inflammation and hepatocytic apoptosis *in vivo*. 1μM and 10μM SA-B improved the apoptotic cell lines' DNA fragmentations, decreased the apoptotic rates by flow cytometry, and mitochondria injury analyzed with Kinetic Scan *in vitro*.
Conclusion: SA-B can effectively suppress hepatocytic apoptosis *in vivo* and *in vitro*.

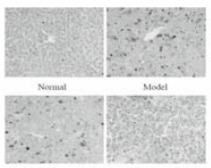

Fig 1. Effect of SA-B on hepatocytic apoptosis *in vivo*. TUNEL ×400

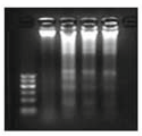

Fig 2 Agarose gel electrophoresis of DNA in SMMC7721 cells N, normal group, M, model group, S1, 1μM SA-B-treated group, S2, 10μM SA-B-treated group.

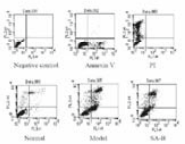

Fig 3 Inhibition of apoptosis by SA-B in SMMC7721 cells, flow cytometry.

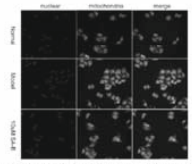

Fig 4 SA-B protects SMMC7721 cells from apoptosis, Kinetic Scan .

PP-039
The value of hyaluronic acid measurement in predicting survival
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Introduction and Aim: Serum fibrosis markers have been compared with liver histology repeatedly in studies which assume the biopsy as the gold standard. Sampling and interpretation variation limits the accuracy and a better, and clinically relevant, reference might be survival. The aim of this study is to examine the value of serum hyaluronic acid (HA) levels in predicting survival in patients with chronic liver disease.
Methods: Single centre retrospective observational study. From an HA database, a cohort of 236 patients with HA values ≥ 100ng/ml was initially derived. We identified those patients who have subsequently died (or underwent liver transplantation (OLT)) and compared HA values with survival (in months) using Spearman, non-parametric correlation.
Results: From the 236 patients, we identified 25 patients (15 M:10F) from 1995 who have subsequently died (or undergone OLT). Mean age was 62.2 years (range of 31-86 years). 4 patients had more than one HA value in excess of 100ng/ml. An inverse correlation of r= -0.411

with a p value of 0.024, was found between the HA and survival in months. Of the 11 patients in this cohort with values > 800, 10 had died within 16 months. Of the 7 patients (8 values) with values < 200, 5 patients survived >16 months. The mean survival for values >800, 601-800, 401-600, 201-400 and 100-200 was 11.6 (11 samples); 27.5(4); 15.5(4); 47.5(2) and 31.1(9) months respectively. Only 3 patients with values greater than 200 survived >30 months.

Conclusion: In this study looking at all cause mortality (or OLT) in a cohort of patients with at least one HA value who subsequently died, HA levels accurately predicted survival in the majority. In particular, levels >800 have a mean life expectancy of less than 1 year in this cohort.

PP-040

Effects of norepinephrine on the proliferation and activation of rat hepatic stellate cells

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Objective: To elucidate the relationship between rat hepatic stellate cells (rHSC) and sympathetic neurotransmitters norepinephrine (NE) during liver fibrosis.

Method: By using immunofluorescence and RT-PCR, we investigated the expression of α_{1A}、β_2-adrenoceptors in activated rHSC; We also adopted the methyl thiazolyl tetrazolium（MTT）to investigate the effect of NE on the proliferation of rHSC; Meanwhile, the expression of collagen-1(Colla-1)、smooth muscle actin-α(SMA-α) and transforming growth factor-β(TGF-β) in NE-stimulated rHSC were detected by RT-PCR. The contents of NE in rHSC were determined by high performance liquid chromatography-electrochemical detector (HPLC-ECD).

Results: The $\alpha_{1A.}$ β_2-adrenoceptors were expressed in rHSC as showed by immunofluorescence and RT-PCR; By using MTT assay, we showed here that the sympathetic neurotransmitter NE markedly stimulate the proliferation of rHSC in a concertration-dependent manner. NE induced the expression of Colla-1、SMA-α and TGF-β in rHSC, as detected by RT-PCR($P<0.05$). Finally, rHSC could also release NE, as detected by HPLC-ECD.

Conclusion: By extending evidence that rHSC are direct targets of the SNS, HSC are hepatic neurogial cells that produced and responsed to sympathetic neurotransmittes norepinephrine, suggesting that interrupting SNS signaling may be useful in liver fibrosis during clinical management. (supported by National Nature Science Foundation of China NO.30571627)

PP-041

Expression of DMBT1 in liver cirrhosis and its relationship with oval cell proliferation

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Objective: To investigate the expression of DMBT1 in liver cirrhosis and its potential relationship with oval cell proliferation. Methods: Immunostaining on serial sections was carried out for CK19 and Hepatocyte to identify oval cells in 22 liver cirrhotic samples. DMBT1 expression in proliferated bile ductules (PBD) was also studied by using monoclonal antibody h12 The homozygous deletion of DMBT1 in PBD was also investigated by laser capture microdissection. Five cases were examined by transmission electron microscope, and 10 normal liver tissues were also used as controls.

Results: Microscopically, oval cells could be found among the reactive ductules in all cirrhotic cases which could coexpress CK19 and Hepatocyte, and its numbers were found to increase significantly with the progression of inflammation. Electron microscope also showed there were oval cells in PBD (Figure1). DMBT1 could express in these oval cells too, and its positive rate in PBD was 45.5% (Figure2). The homozygous deletion of DMBT1 was found in 45.5% PBD which had significantly difference compared to surrounding hepatocytes. PBD and oval cells were not found in normal liver tissues, and the expression of DMBT1 was only detectable in few large bile ducts (Figure3).

Conclusions: There are bipotent oval cells in liver cirrhosis hiding in PBD. DMBT1 expression is upregulated in these cells. DMBT1 can be specific deleted in PBD and comparably consist with its protein expression. DMBT1 may relate to the proliferation of oval cells and may participate in the development of liver cirrhosis.

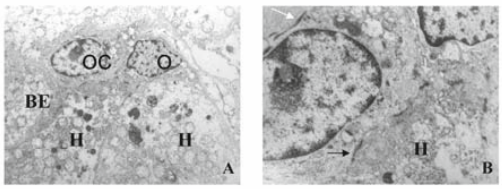

Figure1 A: PBD identified by electron microscopy , which contained two oval cells(OC) with oval shape scant cytoplasm, a biliary epithelial cell (BE) and a hepatocyte (H) ×2000 B: Tonofilaments (black arrow) and intercellular junctions (white arrow) could be seen between the oval cell and the adjacent cell. ×5000

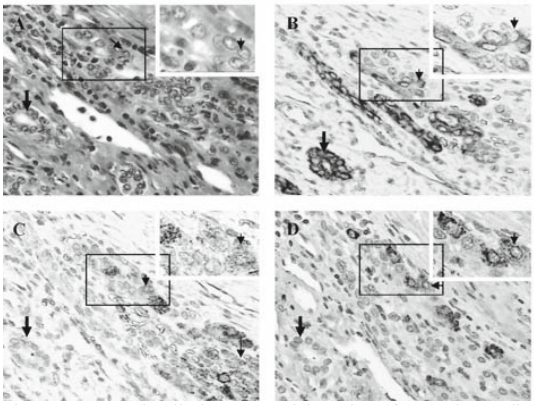

Figure 2: An oval cell shown on serial sections from a human liver cirrhotic specimen. A small bile duct (SBD) is located in the serial sections for orientation purpose (long arrow). This oval cell is differentiating towards hepatocyte with intense expression of Hepatocyte while relatively weak staining of CK19. The boxed areas are magnified to show oval cell morphology in right upside boxes respectively. A, PBD infiltrating lymphocytes can be seen in portal tract. An oval cell with large nucleus and scant cytoplasm are shown. HE. B, This oval cell is identified by CK19 staining. Anti-CK19 (short arrow, red, AEC). C, The oval cell is identified by Hepatocyte staining. AntiHepatocyte (short arrow, red, AEC). D, Serial section shows cytoplasm expression of DMBT1 in this oval cell. Anti-DMBT1. (short arrow, dark brown, DAB).

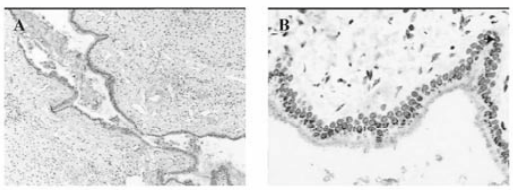

Figure3 A: The expression of DMBT1 in large hepatic bile ducts of normal liver×40
B: DMBT1 is expressed in the supranuclear cytoplasm of biliary epithelial cells ×200

PP-042

Quantitative assessment with contrast-enhanced ultrasonography in a rat model of hepatic fibrosis

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Purpose: Quantitative analysis with contrast-enhanced ultrasonography (CEUS) was used in a rat model of hepatic fibrosis in order to investigate quantitative parameters in the assessment of fibrosis grade.

Materials and Methods: Ninety-two male Sprague-Dawley rats (initial weight: 180-210 g) were administered 10% thioacetamide (TAA) solution via intraperitoneal injection, three times in the first week and twice a week for twelve weeks. CEUS was performed on nine rats once a week since the third week and the liver sample was then removed for histological examination. The dynamic images of CEUS were analyzed with a quantitative software which showed time-intensity curve of the region of interest (ROI). Several quantitative parameters as rate of half

contrast material washout (a2)，peak intensity (PI), area under the curve (AUC), and transit time of hepatic artery to hepatic vein. The fibrosis stages of all the samples were evaluated according to the human standard published by Chinese Medical Association in September 2000. Spearman coefficients were used to assess the correlations between quantitative parameters and the fibrosis stage.

Results: The fibrosis stage on pathology was as the following: S0=11, S1=14, S2=30, S3=27, and S4=10. There were statistical correlations between fibrosis stage and PI (r=-0.663, P<0.001), AUC (r=-0.773, P<0.001), and transit time (r=-0.642, P<0.001) from ROI of hepatic parenchyma. Following the aggravation of liver fibrosis, PI became lower, AUC became smaller and Transit time became shorter.

Conclusion: The quantitative parameters on the time-intensity curve of dynamic CEUS images correlates with the hepatic fibrosis stage in the rat model.

PP-043

Study on the expression of IGFBPrP1 induced TGFβ1 in hepatic stellate cell and the relationship with Collagen I

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Objective: To study the expression of IGFBPrP1 induced TGFβ_1 and the influence of anti-IGFBPrP1 antibody on the production of Collagen I induced TGFβ_1 in HSC.

Methods: Rat HSC-T6 was cultured in vitro and established the control group and TGFβ_1 2ng/ml,4ng/ml ,8ng/ml ,16ng/ml groups. The control groups of anti-IGFBPrP1 antibody ,TGFβ_1 2ng/ml＋anti-IGFBPrP1 antibody 0.1ug/ml ,or 1ug/ml , 2ug/ml groups, or TGFβ_1 4ng/ml＋anti-IGFBPrP1 antibody 0.1ug/ml ,1ug/ml groups. Incubated for 24h , then cell-coated dishes and supernatant were attained or cytoplasmic proteins were extracted , the expression of IGFBPrP1 was detected by both immunocytechemistry staining and Western Blot, and the content of collagen I was measured by ELISA.

Results: The results of both immunocyte-chemisty staining and Western Blot analysis indicated the positive staining of IGFBPrP1 in TGFβ_1 treatment was significantly higher than that in the control group and the positive staining of IGFBPrP1 in TGFβ_1 treatment with 4ng/ml group was the most strong among the total groups. The results of ELISA showed the level of collagen I in TGFβ_1 alone group was significantly higher than that in the control group , the level of collagen I in TGFβ_1+ anti-IGFBPrP1 antibody groups was significantly lower than that TGFβ_1 groups . There was a positive correlation between IGFBPrP1 and collagen I (r=0.833, P＜0.01).

Conclusions: In HSC-T6 the expression of IGFBPrP1 induced TGFβ_1 significantly increased, and anti-IGFBPrP1 antibody can rival the over-production of collagen I induced TGFβ_1. There was a positive correlation between IGFBPrP1and collagen I. IGFBPrP1 involved the information of liver fibrosis and maybe played an important role in the emergence and development of liver fibrosis.

PP-044

Primary study on relationship between heme oxygenase-carbon monoxide pathway and plasma endothelin in portal hypertensive patients

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Objectives: To investigate the relationship between heme oxygenase–carbon monoxide pathway (HO-CO) and endothelin (ET) with portal hypertension in cirrhotic patients and hope to be able to get a method lowering portal hypertension by the intervention of medicine to regulate blood motility.

Methods: Healthy control group (n=20), chronic hepatitis group containing patients with medium or advanced chronic hepatitis (n=20), and cirrhosis group enrolling patients with cirrhositic portal hypertension（n=26）were studyed. Dual-wavelength spectrophotometry was applied to detect the blood COHb concentration representing the level of HO-CO pathway[1], and radioimmunoassay to measure plasma ET-1 level.

Results: 1. COHb concentration of the patient with portal hypertension(1.0285±0.5915%) is remarkably higher than that of the control group(0.495±0.215%) and chronic hepatitis group(0.517±0.293%) (P ＜ 0.05). There is no obvious difference between the latter groups (P>0.05). 2. ET-1 level of the patient with portal hypertension (134.27±14.99ng/ml) is evidently higher than that of control group (44.65±9.08ng/ml) and chronic hepatitis group (63.90±11.93ng/ml) (P<0.05). ET-1 level of chronic hepatitis group is also obviously higher than that of the control group (P<0.05). 3.COHb concentration is positively relevant with ET-1 level in portal hypertension patients (P<0.05), but no obvious relativity showed in chronic hepatitis group.

Conclusions: There may be no obvious relationship between HO-CO pathway and liver fibrostic extent. The plasma ET-1 may have relevance to both of liver fibrostic extent and portal hypertension. The HO-CO and ET-1 levels of patients with portal hypertension are positively relevant. They may work in coordination to sustain the high dynamical circulation of portal hypertension.

[1]DE LAS HERAS D, FERNANDEZ J, GINES P, et al. Increased carbon monoxide production in patients with cirrhosis with and without spontaneous bacterial pritonitis.Hepatology,2003;38:452-459.

PP-045

The regulatory effect of endogenous hydrogen sulfide on portal hypertension

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Objective: To study the changes of endogenous H_2S and the effect of exogenously applied H_2S on portal hypertension (PHT).

Methods: Wistar rats (n=60) were randomly divided into control group，PHT group and PHT+NaHS group. The rats of PHT group was infected with schistosomiasis japonica. NaHS solution was injected to rats at a dosage of 0.78mg/kg everyday. The microstructure and ultrastructure changes in portal vein were examined using Masson staining and transparent electron microscope respectively. The activity of H_2S generating enzymes in homogenates of the hepatic tissue and portal vein was measured. The cystathionine-γ-lyase (CSE) mRNA in hepatic tissue was measured using RT-PCR.

Results: Compared with rats in the control group，the portal venous pressure(PVP) increased 35.8%. The relative medium thickness (RMT) and relative medium areas (RMA) increased 36% and 29% respectively. The ultrastructure of the portal vein also changed significantly. The H_2S generating enzyme activity in hepatic tissue and in portal vein decreased 48% and 55%. There were obviously negative correlations between the PVP and H_2S generating enzymes activity. Relative CSE mRNA amount in hepatic tissue also decreased 46%. The PVP decreased 33% respectively compared with that of PHT group. The RMT and RMA in rats of PHT+NaHS group also decreased 35% and 33% compared with that of PHT group respectively. The H_2S generating enzymes activity and relative CSE mRNA amount in the hepatic tissue increased 53% and 106%.

Conclusion: Endogenous H_2S was involved in the pathogenesis of portal hypertension. Exogenously applied H_2S could exert protective effect during portal hypertension.

PP-046

Distribution of hepatic nerve fibers in cirrhotic mice model induced by carbon tetrachloride: Immunohistochemical and transmission electron microscopic study

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Background/Aim: Intrahepatic innervation plays an important role in the regulation of the hepatic microcirculation as well as the glycogen, lipid metabolism. The aims of this study were to investigate the ultrastructural changes in the mice model caused by the carbon tetrachloride (CCl_4).

Methods: Thirteen of the 8-week-old male C57BL6 mice were intraperitoneally injected with a 50% solution of CCl_4 (2mg/kg) in mineral oil twice a week for 8 weeks. The nerve fibers were immunohistochemically identified using the antibody for the S-100 protein. The electron microscopic study of the hepatic nerve innervation was also performed.

Results: In the control group, the S-100-positive nerve fibers were diffusely distributed within the portal tract. The nerve fiber innervations in CCl_4-cirrhotic group were closer to the ducts and vessels of the portal tract than those of the control group. However, the intralobular innervation was not observed in both groups. Electron microscopic study on the portal tract showed that the hepatic nerves were in contact with the hepatocyte, bile duct, hepatic arteriole, portal venule, and stellate cell. The distances between hepatic nerve fibers and

hepatocyte, hepatic arteriole, and stellate cell were markedly closer in the CCl_4-cirrhotic group compared with the control group, respectively. **Conclusions:** Distributions of the hepatic nerve fibers within the portal tract among the hepatocyte, hepatic arteriole, and stellate cell were markedly closer in the CCl_4-cirrhotic group than in the control group. This study suggests that the hepatic nerve fibers contact became closer in the cirrhotic liver with the development of the liver fibrosis.

PP-047

TGF-β1/ALK1 pathway gene expression in activated hepatic stellate cells and inhibited by herbal compound 861

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Currently, TGF-β1/ALK1/Smad1/5 signaling was found in hepatic stellate cells (HSCs) and involved in transdifferentiation of HSCs. Previous researches showed that compound 861 (Cpd861) could inhibit the activation of HSCs to exert its anti-fibrotic effect. Here we studied TGF-β1/ALK1/Smad1 pathway related genes expression in LX-2 cell line (activated HSC) and the inhibitory mechanism of Cpd861 on the activation of LX-2 cells. Real time-PCR was used to examine the gene expression of *α-SMA* (α-smooth muscle actin, marker of activated HSCs), *ALK1* (activin receptor-like kinase 1), *Id1* (inhibitor of differentiation 1), western-blot to examine phosphalated Smad1 and immunohistochemical analysis to examine α-SMA after treatment with TGF-β1 (5ng/ml) and Cpd861 (0.1mg/ml) on LX-2 cells for 24 hours. It was shown that α-SMA gene increased to 2.65 fold by TGF-β1 versus control and decreased by Cpd861 to 0.38 fold, which was proved by immunohistochemical analysis. Id1 expression was stimulated by TGF-β1 to 2.5-fold and decreased by Cpd861 to 0.53-fold.The level of phosphalated Smad1has the same trends. Effects of Cpd861 still were seen in LX-2 cells co-treated with TGF-β1. ALK1 mRNA was significantly reduced by Cpd861. In conclusion, Cpd861 can restrain the activation of LX-2 cells by inhibiting the TGF-β/ALK1/Smad1 pathway

PP-048

Effects of valsartan on immunodamaged hepatic fibrosis and bcl-2/Bax in rat

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Objective: To study effects of angiotensin Ⅱ type 1 recepter blockade (valsartan) on immunodamaged hepatic fibrosis in rats induced by pig serum and observe its effects on the bcl-2/Bax of the rats' hepatic stellate cells so as to probe the mechanisms of anti-fibrosis.

Method: 36 SD rats were randomly divided into three groups, normal control group, model group and treated group.Experimental models were produced by repeated injections of pig serum. Beginning from the sixth week, the treated group(12 rats)were treated with the same dose of Valsartan(30mg/kg.d) and when the experiment ended in the tenth week, the level of hepatic fibrosis and the serum levels of hyaluronic acid(HA), hydroxyproline(hyp), were observed in the model group ,treated group and control group. The expressions of α-SMA, Bax and bcl-2 were measured by immunochemical techniques.

Results: Rat hepatic fibrosis was developed as showed by histological examination.The hepatic fibrosis and inflammation were significantly alleviated compared with the model group. The area of collagen in the model group was 12.5±2.15% and that in the treated group, 5.21±1.21%. The compasion between the two groups has some sigificane in statistics. Compared with the model group the serum level of HA, hyp was lowered. The result of immunochemical techniques shows that the expression of α-SMA in the model group was greatly increased while that, apparently decreased in the treated group ($P<0.05$). Compared with the control group the expression of bcl-2 and Bax was obviously increased in the model group. The expression of bcl-2 in the treated group was apparantly reduced ($P<0.05$) while the expression of Bax was not greatly changed ($P>0.05$) and the proportion of bcl-2/Bax was decreased.

Conclusions: Angiotensin Ⅱ type 1 recepter blockade (valsartan) significantly delayed the progression of pig serum-induced liver fibrosis in rats and the mechamism may be associated with the reductive expression of bcl-2 and decrement of the proportion of bcl-2/Bax.

PP-049

CT diagnosis of hepatitis induce Early liver cirrhosis of the clinical and pathological control

Min-ning Song[1]
[1] *xiam 174 hospital*

Objective: Analysis of CT in the hepatitis induce early cirrhosis (Child-Pugh A level) diagnostic value.

Methods: CT diagnosis early liver cirrhosis group and the group of chronic hepatitis will be liver biopsy, using hematoxylin (HE), reticular fiber Gomori, Fanjisen (VG), connective tissue Masson trichrome staining pathological examination . Results will be pathological examination and clinical and CT imaging characteristics of contrast.

Results: CT examinations showed 48 cases of early liver cirrhosis, the results of pathological examination with only six cases (6 / 48, 12.5%). Chronic hepatitis, mild pathological diagnosis rate for the 36/42, 85.7%; 36/48, 75%. However, the two groups do not meet only part of the Light - the intersection between moderate. Severe chronic hepatitis six cases, pathological diagnosis with six cases (6 / 6, 100%). CT morphology of the shrinking of the liver imaging characteristics, and indications of liver pathology fibrosis rate was 100%. With no signs of liver narrow the control group, no one is in conformity with.

Conclusion: CT morphology narrow is early liver cirrhosis more precise diagnosis. Chronic hepatitis clinical diagnosis and pathology consistent with the high percentage of non-essential to avoid the high frequency of liver biopsy for diagnosis.

PP-050

A study of endocannabinoids-anandamide involved in the proliferation and inflammatory regulation in rattus hepatic stellate cells

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Background: Endocannabinoids are known to mediate a wide spectrum of central and peripheral effects, including pain alleviation, immunological regulation, anti-inflammatory, anti-tumor and hemodynamics properties. An increasing body of evidences about anandamide (AEA) have indicated its role in the systemic circulation disturbance in advance cirrhosis. Howerve, its precise physiological role in liver fibrosis has not been fully elucidated.

Aim: This study determined, in addition to the direct antifibrogenic effects, whether AEA may also regulate the pro-inflammatory function of rattus hepatic stellate cells (rHSCs) during liver fibrosis.

Methods and results: By using MTT assay, we demonstrated that AEA trigger potently grow inhibition in rHSCs, but not hepatocyte, in a concentration-dependent manner (from 5umol/L -20umol/L), and higher does (40umol/L) were necrosis. Nuclear staining of Hoechst 33258 and Annexin-V/PI binding assay showed that AEA induce rHSCs necrosis but not apoptosis. Whereas the putative cannabinoids receptors antagonism (SR141716A and AM630), failed to block anandamide-induced cell death. In this context, methyl-β-cyclodextrin (MCD)(1mmol/L), a membrane cholesterol depletor potently inhibited the anandamide-induced rHSCs death($P<0.05$). Besides, Anandamide slightly stimulated the secretion of inflammatory cytokines (interleukin-2, 6, tumor necrosis factor-α) in rHSCs ($P<0.05$), as assessed by RT-PCR and ELISA.，showing a pro-inflammatory property. **Conclusion:** AEA could potently suppress the proliferation of rattus hepatic stellate cells via the membrane cholesterol but not putative receptors, and show a pro-inflammatory property during liver fibrosis. (Supported by National Nature Science Foundation of China NO.30571627)

PP-051

A study on the mechanism of anti-human hepatic fibrosis with Chinese medicinal herbs, Fufangbiejiaruanganpian

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Objective: To study the mechanism of action of anti-human hepatic fibrosis with Chinese medicinal herbs, *Fufangbiejiaruanganpian (FFBJRGP)*.

Materials and Methods: The pre- and post- treatment biopsy liver specimens and whole clinical data from 65 cases of patients with chronic hepatitis B, treated with FFBJRGP for 1/2~1years, were investigated.

Results: Before and after-treatment histological fibrosis scoring evaluation showed the effective rate for 65 cases of subjects was 81.5%.

The histological assessments indicated that after the *FFBJRGP* treatment for more than six months, the obviously down-regulated TIMP-1 and TIMP-2 expression, as well as up-regulated MMPs and MT-MMPs expression at protein and mRNA levels were found in the liver tissues. Compared with pre-treatment, post-treatment immunostaining scores for α-SMA and PCNA expression in hepatic stellate cells were much lower, while the number of stellate cell apoptosis increased. After *FFBJRGP* therapy, the expression of TGF-β1 and IL-1β were suppressed at protein and mRNA levels. According to degradation degrees after treatment, the ECM component degradation in order was collagen III, IV, laminin, fibronectin, and collagen I.

Conclusion: The results suggested that *FFBJRGP* might affect several key steps in the process of hepatic fibrosis and possess multi-drug effect targets for anti-hepatic fibrosis.

PP-052

Quantitative assessment of mRNA TGF-β1 in liver tissue in connection with serum mean daily level of TGF-β1 in chronic hepatitis B patients

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Monitoring of fibrosis process with the use of histopathologic studies on liver's bioptates is limited, so it is attempted to find reliable, obtained with less invasive methods, sensitive and reflecting fibrosis dynamics markers of this process. The aim of the study was simultaneously to assess liver's expression as well as circadian and mean daily TGF-β1 concentration in serum in patients with chronic hepatitis type B in comparison with control group. Studies were performed on 50 patients (9 women, 41 men) aged 45.9 ± 2.3 years with chronic hepatitis type B. Control group consisted of 20 patients (mean age 38.6 ± 3.7 years), in which so called minimal changes without fibrosis were observed in histophatologic bioptate of liver. Blood for studies was collected every 4 houres during the day. Concentration of TGF-β1 in serum was assessed with the use of ELISA method and expression of mRNA TGF-β1 in liver with QRT-PCR method. No significant difference between circadian as well as mean daily serum TGF-β1 concentration beetwen control group and the group with chronic hepatitis type B was shown. Increased expression of mRNA TGF-β1 in bioptate of liver of patients with chronic hepatitis type B in comparison with control group was noted. In "minimal changes" control group presence of significant positive correlation between expression of mRNA TGF-β1 in liver and concentration of this cytokine in serum was shown, in the group of patients with chronic hepatitis B this connection was not noted. Results of the study suggest, that expression of mRNA TGF-β1 in liver biopsy specimens seem to be usefull prognostic marker in patients with chronic hepatitis type B.

PP-053

Serum aminoterminal peptide of type III procollagen (PIIINP) and transforming growth factor-beta1 (TGF-beta1) levels in patients with chronic hepatitis B and C

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Monitoring fibrosis process by liver's biopsy is limited, so many attempts are undertaken to assess concentrations of definite proteins in blood, which could be easily accessible marker of intrahepatic process. It seems that among others, detrminations of blood concentration of PIIINP – index of collagen's III synthesis and TGF-β1 – cytokine of antiproliferative action and inhibiting hepatocytes' growth, yet inducing fibroblasts' growth and stimulating fibrosis process brings out such a possibility. The aim of the study was to simultaneous determination of TGF-β1 and PIIINP concentration in blood of 40 patients with chronic hepatitis B (CHB) and 35 patients with chronic hepatitis C (CHC) in comparison to healthy controls. Results: Increased serum concentrations of TGF-β1 as PIIINP in both groups of patients (CHB and CHC; grading 2-3, staging 1-2) in comparison with control group was noted. Positive correlation of TGF-β1 and PIIINP serum concentrations in both groups of patients was observed. There was no changes in PIIINP serum levels in CHB and CHC patients in dependence on stage of liver fibrosis (staging 1 vs staging 2) but TGF-β1 was increased in CHB and CHC patients with higher stage of liver fibrosis process. On the base of obtained results, it seems that changes in TGF-β1 concentrations in blood reflect "grading" and "staging" whereas PIIINP levels in blood have rather the relation with "grading".

PP-054

Effect of pentoxifylline on carbon tetrachloride induced chronic liver injury

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Background: Hepatic fibrosis is one of the processes that occur when the liver is damaged through viral activity, toxins, autoimmune diseases, metabolic disorder or genetic defects. It is a result of chronic liver injury that ultimately leads to cirrhosis.Efficient and well tolerated anti-fibrotic drugs are lacking. In search for novel drugs that can alleviate hepatocyte injury and reduce fibrosis, Pentoxyfilline (PTX) has received considerable interest.

Aim: To study the effect of Pentoxyfilline on chronic liver injury using carbon tetrachloride (CCl_4) induced liver injury animal model.

Methods & Materials: Wistar rats were divided into 3 groups: normal control group (Group I),CCl_4 induced liver injury control group (Group II) and CCl_4 induction with Pentoxyfilline treatment group (Group III). Liver damage and fibrosis was induced by subcutaneous injection with 40% CCl_4.Group III also received daily intra peritoneal injection of Pentoxyfilline.Degree of fibrosis were measured and serological markers for liver fibrosis and function including hyaluronic acid (HA), type IV collagen (CIV), γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined. Alpha smooth muscle actin (α-SMA) and proliferating cell nuclear antigen (PCNA) immunohistochemistry were also performed.

Results: CCl_4 induction led to the damage of liver and development of fibrosis in group II and III rats when compared to group I rats. The treatment of Pentoxyfilline in group III rats could reduce the fibrosis condition significantly compared to group II rats. Serum HA, CIV, ALP, ALT, AST and GGT levels after treatment were significantly different from CCl_4 induced liver injury control group but similar to the normal control group. An increase in PCNA and decrease in α-SMA expression level was also observed.

Conclusions: Pentoxyfilline could improve liver function and reduce liver fibrosis which might be through the inhibition of hepatic stellate cell activity.

PP-055

Effects of valsartan on apoptosis of hepatic stellate cell in rats with liver fibrosis

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Objective: To evaluate the effects of angiotensin Ⅱ type 1 receptor (AT_1R) blockade on proliferation and apoptosis of hepatic stellate cell (HSC) in immunodamaged hepatic fibrosis rats.

Methods: 36 healthy S-D rats were randomly divided into 3 groups: group A:12,normal control; group B:12, model control;group C:12,administrated orally with valsartan (30mg/kg) daily via gastrogavage.Group B and C were induced by repeated intraperitoneal injection with pig serum twice every week. The degrees of inflammation and fibrosis were evaluated by HE and Sirius red stainins. Alpha-SMA, Bax and bcl-2 were detected by the immunohisochemistry staining. Apoptosis of HSC was detected by double-stainings of terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) and alpha-SMA immunohisochemistry stianing.

Results: As compared with group B, in group C, the degrees of fibrosis were significantly attenuated (5.16±2.10 *vs* 10.08±2.01, $P<0.01$); the activated HSC were reduced (7.53±1.58 *vs* 15.38±3.87, $P<0.05$); The expression of bcl-2 was in down-regulation (3.67±1.27 *vs* 6.73±2.19, $P<0.05$) and the number of HSC of apoptosis was increased (4.75±2.01 *vs* 1.8±1.14, $P<0.05$).

Concluzions: AT_1R blockade can release immuodamaged liver fibrosis, inhibit activation and proliferation of HSC and induce apoptosis of HSC by down-regulation of bcl-2.

PP-056

Activation of human hepatic stellate cell line, LX-2 by hepatitis B X gene transfection

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Liver cirrhosis is one of the major complications of hepatitis B virus (HBV) infection, but the mechanisms underlying HBV-related fibrogenesis are still not clear. Although the roles of hepatitis B virus X protein (HBx) remain poorly understood, it is supposed to play an important role in the regulation of cellular growth and hepatocarcinogenesis. The aim of this study was to examine the role of HBx on the hepatic fibrogenesis. We established a LX-2 cell line expressing HBx protein by stable transfection. Cell proliferation and cell cycle assay revealed that HBx could promote cell proliferation and accelerate the progression of G1 to S in cell cycle of LX-2 cells. The expressions of fibrosis-related molecules transforming growth factor -β1 (TGF-β1), transforming growth factorβ receptor II (TGFβRII), α-smooth muscle actin (α-SMA) and connective tissue growth factor (CTGF) were analyzed *via* Western blot and or semi-quantification RT-PCR **.** In addition, the expression levels of collagen type I (Col I) from the media in LX-2 cells were measured by ELISA. The expressions of α-SMA, TGF-β1,Col I, TGFβRII and CTGF were significantly increased in LX-2 /HBx cells expressing HBx protein **.** These results suggest that HBx protein may contribute to the hepatic fibrogenesis *via* promotion of HSC proliferation and up-regulation of TGF-β1 and CTGF.

PP-057

CB2 cannabinoid receptor antagonist, AM 630, ameliorated hepatic oxidative stress in bile duct ligated mice

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The endocannabinoid system is an important regulator of hepatic fibrosis associated with chronic liver diseases. Cannabinoids are the active components of marijuana and act via 2 G-protein coupled receptors, CB1 and CB2. Here, we investigated whether activation of cannabinoid CB2 receptor promotes progression of liver injury.

Hepatic injury was induced in female Hamster mice by bile duct ligation (BDL). Controls underwent sham operation. At 4 days after surgery, mice were receiving AM630 (1 mg□kg-1of BW□ day-1, intra-peritoneal injection), a CB2 antagonist, for 3 consecutive days. At 7 days after operation, blood and liver tissue were harvest for further analysis.

Immunohistochemistry show induction of CB2 receptor located within in α-smooth muscle actin positive cells in liver sample following BDL surgery. AM630 decreased CB2 protein and mRNA expression by Western blot and reverse transcriptase polymerase chain reaction. CB2 activation triggered damage involved iNOS, COX-2 protein and macrophage infiltration in hepatic tissue. Pharmacological inactivation of CB2 receptor decreased fibrogenesis by lowering hepatic α-SMA. AM630 attenuated hepatic injury also involved lipid pathways, which were accompany by a remarkable increased hepatic GSH/GSSG ratio as well as SOD activities in BDL-induced hepatic injury. Finally, proteomics approaches show the major effects of AM630 was evident up-regulation of regucalcin, selenium-binding protein, chaperone, protein disulfide isomerase protesomes in liver tissue of BDL mice.

These data highlight the hepatoprotection role of CB2 receptor antagonist, AM630, during chronic liver injury.

PP-058

Quantitative analysis of liver fibrosis with contrast-enhanced ultrasonography: compared with biochemical tests

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Objective To investigate the relationship between quantitative parameters of contrast-enhanced ultrasonography (CEUS) and histological hepatic fibrosis stage in rat model, and compared with the results of routinely available biochemical test.

Methods Ninety-two male Sprague-Dawley rats were administered 10% thioacetamide solution twice a week for 12 weeks intraperitoneally. CEUS was performed on nine rats every week since the 4th week. Then, the rates were killed for blood test and histological examination of liver sample. The dynamic images of CEUS were analyzed quantitatively from the time-intensity curve of liver parenchyma, and provided some parameters as the increase signal intensity (ISI), rate of half contrast material washout (a2), peak intensity (PI), area under the curve (AUC), and mean transit time (MTT). The fibrosis stages were evaluated according to the human standard published by Chinese Medical Association in 2000. Spearman coefficients were used to assess the correlations between CEUS parameters and fibrosis stages.

Results The fibrosis scores on pathology were S0 (n=11), S1 (n=14), S2 (n=30), S3 (n=27), and S4 (n=10). There were statistical correlations between fibrosis stage and CEUS parameters as ISI (r=-0.508), a2 (r=0.448), PI (r=-0.663), AUC (r=-0.773), and MTT (r=-0.642) from liver parenchyma. Also, statistical correlations were found between fibrosis and total bilirubin (r=0.422), direct bilirubin (r=0.431), γ-GT (r=0.407), and total bile acids (r=0.505) (P<0.001). There were statistical differences between different fibrosis grades and CEUS parameters as PI, AUC and MTT (P<0.01). However, statistical differences of laboratory test were only found between fibrosis grade S0 and grade S4 (P<0.01).

Conclusion The noninvasive imaging method CEUS is potential in liver fibrosis staging and superior to biochemical tests in rat model.

PP-059

Dynamic changes of adiponectin during hepatic stellate cell activation and its effects on expression of hepatic extracellular matrix

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Aim: There is increasing interest in the influence of adiponectin, a member of adipokines on the liver fibrosis. To investigate the relationship between adiponectin and liver fibrosis, we examined the dynamic changes of gene expression of adiponectin during the transdifferentiation of hepatitic stellate cell (HSC) and the expressions of Matrix metalloproteinases-13 (MMP-13), Matrix metalloproteinases-2 (MMP-2), Tissue inhibitor of metalloproteinase-1 (TIMP-1) and Collagen-1 (COL-1) by exogenous adiponectin stimulation.

Methods: Primary HSCs was isolated from the liver of male Wistar rat. MTT colorimetric array was used to study the influence of adiponectin on the proliferation of HSCs. After treated with adiponectin, the mRNA expressions of hepatic extracellular matrix in HSCs were detected by real-time PCR, and the protein levels in the medium were examined by enzyme linked immunosorbent assay (ELISA).

Results: The survival rate of HSCs negatively correlated with adiponectin concentration (r = 0.828, $P < 0.05$). Expression of adiponectin was down-regulated during the transdifferentiation of HSCs. The mRNA and protein expressions of MMP-2 and MMP-13 were increased to 2.19 and 2.63 ($P<0.05$) fold at lowest dose of adiponectin, with the effects being seen in a dose dependent manner, whereas TIMP-1 and COL-1 expressions were decreased to 39% and 75% ($P<0.05$) respectively.

Conclusion: Adiponectin can inhibit proliferation of HSCs and regulate genes and protein expressions of hepatic extracellular matrix, which maybe its potential anti-fibrotic mechanism.

PP-060

FuzhengHuayu recipe reverses rat liver fibrosis by counteracting TGF-β1 signaling

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Background and Objectives: FuzhengHuayu (FZHY) Recipe is a SFDA approved anti-fibrotic medicine in China. We investigate the mechanism of FZHY Recipe action against liver fibrosis relating to TGF-β1/Smads signal transduction.

Methods: The rats were divided into normal, model and FZHY groups. Liver fibrotic model was induced by DMN. FZHY group rats took

4.6g/kg FZHY recipe for 4w. The hepatic Hyp content was assayed with Jamall's method; Protein expressions of TGF-β1, TGF-β receptor I (TβR-I), TβR-II, SARA and Smad2 were analyzed by Western Blot. Protein expression and location of α-SMA and Smad3 were observed by Western Blot and immunohistochemistric stain.

Results: Model rats increased hepatic Hyp contents, and unregulated TGF-β1, α-SMA and TβR-I expression. Compared to model group, FZHY decreased hepatic Hyp; down-regulated α-SMA, TGF-β1 and TβR-I expressions; and inhibited the nucleus expression of Smad3 in liver tissue.

Conclusions: FZHY could reverse liver fibrosis in rats via inhibition of TGF-β/Smads signal transduction.

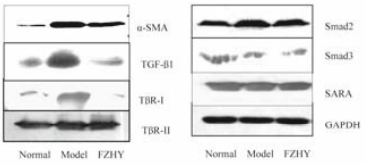

Fig1. The effects of FZHY on expressions of α-SMA, TGF-β1, TβR-I, TβR-II, Smad2, Smad3 and SARA in liver (Western Blotting).

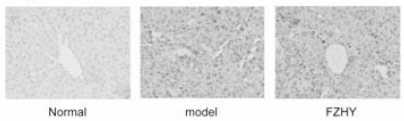

Fig 2. The effect of FZHY on Smad3 expression in liver, immunohistochemistry stain (DAB).

PP-061

Mechanism research of Dangguibuxue decoction on hepatic lipid peroxidation in rats

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Objective: To explore the mechanism of Dangguibuxue decoction on liver fibrosis relating to hepatic lipid peroxidation in rats.

Methods: Liver fibrosis was induced by injection of carbon tetrachloride (CCl_4) subcutaneously and fed with high lipid and low protein diet until 6th weeks. Treated group were administered with Dangguibuxue decoction (DGBXD) at the dose of 6g/kg. Hepatic inflammation was stained with HE and Collagen deposition with Sirius red. The expression of a-SMA was analyzed by immunohistochemistry and Western blotting. Gelatinase activity assay was measured with gelatinozymography and situ zymography.

Results: Compared with model rats, treated group rat showed slighter hepatic collagen deposition, and better liver lipid peroxidation level while α-SMA expression was reduced apparently. Matrix metalloproteinase (MMP2/9) were decreased distinctly especially of active MMP-2.

Conclusion: DGBXT has a good effect against liver fibrosis, which may be related to the prevention from lipid peroxidation.

PP-062

The expressing profile and role of Wnt signal pathway in experimental liver fibrosis on rats

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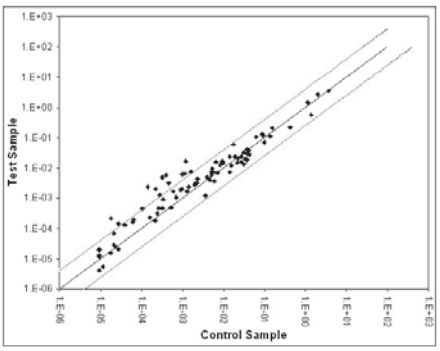
Objective: To study the expressing profile of Wnt signal transduction pathway and explore its role in the experimental model of liver fibrosis.

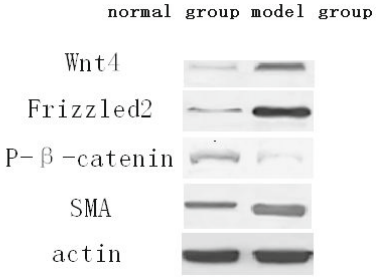
Methods: Experimental model was induced with carbon tetrachloride. Wnt real-Time PCR array was performed. Up-regulated or down-regulated genes were selected if the fold-changes were greater than 2 or less than 2.Expressions of SMA、Wnt4、Frizzled2 andβ-catenin were examined by immunohistochemistry and western blot.

Results: Our analysis revealed 25 genes were upregulated and 11 genes were downregulated remarkably.In experimental group,Wnt4、Wnt5a and Wnt11 were upregulated 13.9、16.5 and 2.17 fold respectively, Wnt1 and Wnt3 were downregulated 2.32、2.15 fold respectively . Expressions of SMA、Wnt4 and Frizzled2 were markedly higher than those in normal group. While the level of phosphorylatedβ-catenin was decreased in model group.

Conclusion: Both canonical and noncanonical Wnt signal transduction pathway are stimulated in experimental liver fibrosis and may participate the mechanisms of liver fibrogenesis.



Scatter plot of the Wnt signal pathway expressing profile



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PP-063

Correlation between metallothionein and the expression and the activity of matrix metalloproteinase-2 in liver

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Objectives: To investigate the relevance between metallothionein and matrix metalloproteinase-2 (MMP-2) expression and activity in mouse hepatic stellate cells (HSCs).

Methods: Fifty Kunming male mice, weighing 25±5g, were randomly divided into two groups, including experiment group (40 mice) and control group (10 mice). The hepatic fibrosis model was induced by CCl_4 in the experiment group. Conditioned medium of HSCs (containing MMP-2), adding different concentrations of MT, then MMP-2 activity was detected. Live tissue microarray was done. Collagen fibers were detected by Sirius red staining. The expression of MMP-2 and MT protein in liver tissues were detected by immunohistochemistry. Gel zymogrphy was used to confirm the activity of MMP-2 in liver tissue homogenate and in the conditioned medium.

Results: The liver tissues after Sirius red staining showed that collagen deposition in the experiment group increased gradually and formed thin to thick fiber septa with the increase of experimental time.The expression of both MMP-2 and MT protein of liver tissues increased wave upon wave in the process of fibrogenesis in the model mice alternately, but the proteins shew out of phase changes. The activity of MMP-2 in the liver tissues also increased gradually and fluctuated with the development of fibrosis. Apart from individual time points, the activity of MMP-2 and MT expression were negatively correlated. The activity of MMP-2 in the conditioned medium treated by MT declined in dose dependent manner($r=-0.9990, P<0.01$).

Conclusions: Our findings suggest that there is an upward tendency in the expression of MMP-2 and MT proteins in the liver fibrogenesis, but interaction between them may be exist, and MT can inhibits the activity of MMP-2 *in vitro*. Further study is needed for the specific mechanisms.

Supported by Natural Science Fundation of China(30470780) ,Corresponding Author: Pingsheng Chen

PP-064

Research on patterns of hepatic cells death during hepatic ischemia-reperfusion injury in cirrhotic rats

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Objective: To research on the main pattern of hepatic cells death during hepatic ischemia-reperfusion(I/R) injury in cirrhotic rat.

Methods: Primary method involves implementation of carbon tetrachloride replication to duplicate a model of cirrhotic rats, and then randomly group them into Sham group and I/R group. The I/R group is further divided into five subgroups: 0 h, 1 h, 6 h, 24 h, 48 h. The samples of livers will undergo several biochemical comparisons, including serum ALT/AST level, the Na^+-K^+ ATPase, Ca^{2+}ATPase, flow cytometry observing percentage of apoptotic/ oncotic hepatic cells, and observation of changes in hepatic cellular structures under transmission electron microscope.

Results: In comparison to the Sham group, the I/R group shows a significant increase in the level of serum AST and ALT, with the peak at 6 hours followed by gradual declination. The active levels of the Na^+-K^+ ATPase and Ca^{2+}ATPase dramatically decrease to minimum one hour after reperfusion treatment and later with a recovery. Hepatic cells suffer oncosis in 6 hours of the early period, while they suffer apoptosis at late stage around 24 hours after reperfusion. Typical appearances of oncosis and apoptosis can be observed.

Conclusion: Oncosis is the main pattern of hepatic cells death during I/R injury in cirrhotic rat, the severity of hepatic injury correlates with the oncosis.

PP-065

Oligodeoxynucleotide as a potential therapeutic agent for liver cirrhosis

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The extracellular matrix (ECM) is composed of several families of macromolecular components: fibrous proteins such as collegens, fibronetin, elastin, and glycocojugates. Type I collagen (COL1) is the major fibrous collagen of bone, tendon, and skin; while type III collagen (COL3) is the more pliable collagen of organs like liver. Fibrosis is common in the establishment of benign tumors and cancers. Evidence has demonstrated that liver, which undergoes fibrosis, also become cancerous. The consensus TGF-β element (TGCCCACGGCCAG) located at approximately -1610 bp from the start site of transcription of the rat proα1 (I) collagen gene has recently been shown to be required for basal promoter activity of this gene. Antisense oligodeoxynucleotides (ODNs) provide a novel therapeutic strategy to regulate gene expression. This therapy provides precise and effective modification of specific gene expression. A novel approach to regulate the activity of collagen genes, thereby affecting fibrosis, is to use ODNs having same sequence of the cis element which regulates the promoter activity of proα1(I) collagen gene. Our laboratory has shown that ODNs containing the TGF-β regulatory element of proα1 (I) collagen gene regulated the transcription of this gene and directly inhibited collagen synthesis in fibroblasts in vitro. Hepatocellular carcinoma is associated with liver fibrosis. Murine schistosomiasis infection offers a model to study the molecular mechanisms of ODNs containing the TGF-β regulatory element in suppressing hepatic fibrosis in vivo. Gel mobility shift analysis showed ODNs containing the TGF-β regulatory element prevented binding of the TGF-β activator protein to the TGF-β regulatory element. It is also successful therapeutic agent to inhibit hepatic fibrogenesis and associated hepatocellular carcinoma.

PP-066

Antifibrogenic effect of 15-Deoxy-Δ12, 14-prostaglandin J2

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We previously showed the antifibrogenic properties of 15-d-PGJ2 in vitro. Here, we investigate this effect in vivo. Male C57 mice were subject to repeat intraperitoneal injections of 10% CCl4 (CCl4/olive oil, 10ml/kg) and 15-d-PGJ2 (0.2mg/kg) twice weekly for 4 or 8 week. CCl4-induced chronic inflammation were not attenuated by15-d-PGJ2. CCl4-induced hepatic fibrosis was markedly ameliorated by 15-d-PGJ2. 15-d-PGJ2 decreased the mRNA expression of collagen type Ⅲ/Ⅰ, α-SMA, TIMP I in liver of CCl4 treated mice. Immunohistochemistry showed that α-SMA and PCNA double-positive cells markedly decreased in 15-d-PGJ2 treated mice. In conclusions: 15-d-PGJ2 exerts preventive effect on CCl4- induced hepatic fibrosis in mice. The mechanism underlying this effect involves both inhibiting the proliferation of AHSCs and reducing the production of TIMP Ⅰ.

PP-067

Effect of transplanting IL-10 gene-modified bone marrow- derived liver stem cells on chronic liver injuries in rats

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Bone marrow stem cells (BMSCs) transplantation alone may not be sufficient for treating chronic liver injuries because of indeterminateness of BMSCs differentiating into hepatocytes and complicated histopathological changes in the liver such as local inflammation and excessive extracellular matrix deposition. Gene therapy strategies offer the potential to prevent graft rejection or inflammatory response, and combining genetic modification of donor tissue with complementary changes may benefit to transplantation. Since BMSCs may be a potentially useful vehicle for gene delivery in adult stem cell-based gene therapy, an alternative strategy could involve transplantation of BMSCs transduced with an adenovirus-mediated anti-fibrosis gene. β_2m^-/Thy-1^+ bone marrow-derived liver stem cells (BDLSCs) possess characteristics of both stem and liver cells. Interleukin-10 (IL-10) is an anti-fibrosis cytokine. BDLSCs modified by IL-10 gene may be useful for treating chronic liver injuries. To determine effect on chronic liver injuries in rats by transplanting BDLSCs transduced with adenovirus-mediated IL-10 gene (AdIL-10), rat BDLSCs were isolated by magnetic bead cell sorting, transduced with AdIL-10, and transplanted into rats with chronic liver injuries. We show that transplanting AdIL-10-transduced BDLSCs into chronic liver injuries rats via the portal vein upregulated IL-10 level in liver, promoted liver regeneration and improved liver histopathology and liver function. These findings demonstrated the potential utility of this novel combined strategy of IL-10 gene and BDLSCs for treatment of chronic liver injuries.

Poster Session – Liver Cancer

PP-068

The predictive role of the metastasis-related miRNAs signature for prognosis of patients with hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most fatal cancers with high risk of metastasis and recurrence. MicroRNAs (miRNA) are a class of naturally occurring small, non-coding RNA that play an integral role in gene expression and regulation. miRNAs have also been implicated in various aspects of human disease, including cancer and its metastasis. In the previous microarray analysis, we have identified a signature of 20 miRNAs that can predict HCC patients with their propensity to metastasize. To further validate the microarray results and optimize the combination of miRNA signature, we investigated the expression of these candidate miRNAs (Let-7g,miR-207,miR-219-1,miR-338,miR-34a,miR-30c-1,miR-148a, miR-148b,miR-122a,miR-15a,miR-9-2) in HCC tissues from an independent cohort of 32 patients with HCC, and a series of of HCC cell lines with different metastatic potential(MHCC97-L,HCC97-H, HCCLM3, HCCLM6) using Taqman real-time RT-PCR. We found that, although not all of the 11 miRNAs have a statisitically significantdifference between the metastasis and non-metastasis case, part of them are closely related to the patients' overall and disease-free survivals. miR-9-2 has a higher expression level in HCC tissues comparing with the corresponding non-cancerous liver tissues, while the miR-122a expression level is lower. The results will be further confirmed by an addition of sample size. Our study will present more information about the 20-miRNAs signature which may add independent prognostic information to clinicopathologic risk assessment for patients with early HCC. And the following functional study may help to elucidate the mechanism leading to HCC metastasis to some extent.

PP-069

Expression of transcription factor KLF8 in HCC and its significance

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Kruppel-like factor 8 (KLF8) is a member of Sp/KLFs transcription factor family. Recent studies indicated that KLF8 participates in v-Src-induced oncogenic transformation and induces Epithelial to Mesenchymal Transition (EMT). Its expression and role in human liver and Hepatocellular carcinoma (HCC) development and progression remain unclear. Using Quantitative real-time PCR, we investigated KLF8 expression patterns in different liver cell lines: L02, HepG2, SMMC7721, SF/SMMC7721, MHCC97L, MHCC97H, HCCLM3. KLF8 mRNA level is increasing with increased invasion and metastasis potential of liver cancer cells. Immunocytochemical analysis showed that KLF8 protein expressed predominantly in the nuclei of the cancer cells. Western blot results showed KLF8 protein level in HCCLM3, MHCC97L is higher than that in L02 and HepG2. Using immunohistochemistry, we investigated KLF8 expression patterns in 33 cases Hepatocellular carcinoma (HCC), 6 cases metastatic tumor, 6 cases normal human liver and 6 cases liver cirrhosis tissue specimens. Overall, metastatic specimens exhibited the highest level of KLF8 expression (83.3% strong positive; $P < 0.001$), and primary tumor tissue specimens had higher levels of KLF8 expression than normal and liver cirrhosis tissue specimens ($P < 0.001$). KLF8 mRNA level in SF/SMMC7721 (c-Met transfected SMMC7721) is higher than that in SMMC7721, indicates a possible relationship between HGF and KLF8 overactivation. All of these suggest that abnormally activated KLF8 expression may directly contribute to Hepatocellular carcinoma development and progression. It can become a potential molecular marker for poor prognosis and target of therapy.

PP-070

Genetic classification in liver fluke associated intrahepatic cholangiocarcinoma based on array-comparative genomic hybridization

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Genetic instability resulted from chromosomal aberrations has been observed in several of solid tumors and is a major mechanism in cancer development and progression. The instability may occur in cancer-related genes involving in cell cycle regulation, apoptosis, cell proliferation and differentiation, angiogenesis and metastasis. Our study attempted to classify genetic alterations in liver fluke associated intrahepatic cholangiocarcinoma (ICC) for better understanding of its pathogenetic mechanisms. Sixty human ICC including 7 adenomas [intraductal type (ID)], 53 ICCs [38 mass forming type (MF) and 15 ID] were analyzed for whole-genomic alterations using array-comparative genomic hybridization (aCGH). Tumor and reference DNA were differentially labeled and hybridized on arrays which consist of 2,440 bacterial artificial chromosome (BAC) clones spotted in triplicate with high resolution 1.4 Mb. Genetic alterations defined by chromosomal aberrations either gains and/or losses of part of the genome or multiple amplified and deleted regions. Hierarchical clustering analysis of genetic alterations was performed and analyzed in each category of subtype of ICCs and adenoma. Our results showed that liver fluke associated ICCs involved multiple chromosomal aberrations, i.e. losses, gained, and amplification. We also focused on specific chromosomal region with aberrations to identify putative gene involvement. This study was the first report of genetic classification in ICCs which was previously undefined. These cancer genetic profiles provide baseline data of ICCs and may be of value in clinicopathological association and discovering of novel cancer related-genes involving in the carcinogenesis of liver fluke associated ICC.

PP-071

Significance of proline rich tyrosine kinase 2 (Pyk2) in modulation of cell motility

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Background and Objective: Proline rich tyrosine kinase 2 (Pyk2) is over-expressed in hepatocellular carcinoma (HCC) and contributes to poor prognosis. In the current study, the role of Pyk2 on cell motility and induction of epithelial to mesenchymal transition (EMT) is being investigated.

Materials and Methods: To study the role of Pyk2 in EMT, Hep3B was forced to express full length Pyk2. In cell line with high endogenous Pyk2 expression (MHCC97L), Non-kinase region of Pyk2 (PRNK) was transfected to suppress Pyk2 activation. Stable transfectants was isolated by G418 selection. Cell morphology and cell motility was examined by scanning electron microscopy and phalloidin staining, respectively. The effect of Pyk2 over-expression on small GTP-binding proteins (Rac1/RhoA) activation was investigated by pulldown assays. Immunoprecipitation was done to study the effect of Pyk2 activation on Hic-5, which associated with EMT.

Results: Transfection of Pyk2 in Hep3B cells resulted in a fibroblastoid phenotype as compared to an epithelioid phenotype in the Hep3B-vector cells. E-cadherin is significantly down-regulated together with up-regulation of mesenchymal genes N-cadherin, fibronectin and TWIST1. More activated Rac1/RhoA were present in Hep3B-Pyk2 transfectants. Transfection of PRNK in MHCC97L cells resulted in fibroblastoid to epithelioid phenotype transformation. E-cadherin expression was restored with distinct adherence junctions. Mesenchymal genes N-cadherin, fibronectin and TWIST was down-regulated as a result of PRNK transfection in MHCC97L cells. Transcription factor STAT5b was also inactivated in MHCC97L-PRNK cells.

Conclusion: Over-expression of Pyk2 may contribute to an epithelial-mesenchymal transition. The induction of EMT could be reversed by suppression of Pyk2 activation.

PP-072

Expression of X-linked inhibitor-of-apoptosis protein in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. Despite significantly improved diagnosis and treatment in recent years, the long-term therapeutic effect is compromised by the frequent recurrence and metastasis, of which the molecular mechanisms are not fully understood. Our initial studies in established HCC cell lines with different metastatic capabilities indicated a correlation of metastasis with the resistance to apoptosis and therefore the ability to survive in stressed conditions. Subsequent investigation revealed that differential expression of X-linked inhibitor-of-apoptosis protein (XIAP) could contribute to the resistance to apoptosis, the ability to grow independently of anchorage, the increased invasiveness in vitro and the enhanced metastasis in vivo. Furthermore, we found that nearly 90% of clinical HCC samples expressed high levels of XIAP. Patients with XIAP-positive tumors had a significantly increased risk to develop recurrent tumors, which was derived from prior tumors as the result of metastasis, following total liver resection and orthotopic liver transplantation. Indeed, XIAP expression can be an independent prognostic factor to predict disease-free survival rate and total survival rate of these patients. Conclusion: our studies have revealed an important molecule in controlling HCC metastasis, defined a molecular marker that can be utilized to predicate HCC recurrence and patient survival following treatment, and suggest that XIAP can be a molecular target subject to intervention to reduce metastasis and recurrence.

PP-073

MiRNA expression profiling reveals metastasis-related microRNAs in hepatocellular carcinoma

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MicroRNAs (miRNAs) have been used as cancer-related biomarkers. Hepatocellular carcinoma (HCC) is an aggressive cancer with a dismal outcome largely due to metastasis and postsurgical recurrence. We investigated whether the expression of certain miRNAs are associated with HCC metastasis. We examined the miRNA expression profiles of 482 cancerous and noncancerous specimens from radical resection of 241 patients with HCC. Using a supervised algorithm and a clinically well-defined cohort of 131 cases, we built a unique 20-miRNA metastasis signature that could significantly predict ($P < 0.001$) primary HCC tissues with venous metastases from metastasis-free solitary tumors with 10-fold crossvalidation. However, significant miRNAs could not be identified from the corresponding noncancerous hepatic tissues. A survival risk prediction analysis revealed that a majority of the metastasis-related miRNAs were associated with survival. Furthermore, the 20-miRNA tumor signature was validated in 110 additional cases as a significant independent predictor of survival (P _ 0.009) and was significantly associated with both survival and relapse in 89 cases of early stageHCC (P_0.022 and 0.002, respectively). These 20 miRNAsmay provide a simple profiling method to assist in identifying patients with HCC who are likely to develop metastases/recurrence. In addition, functional analysis of these miRNAs may enhance our biological understanding of HCC metastasis.

PP-074

X-linked inhibitor of apoptosis-associated factor 1 expression is significantly reduced in hepatocellular carcinoma

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Background: X-linked inhibitor of apoptosis (XIAP) is the most potent member of the IAP family that exerts antiapoptotic effects. Recently, XIAP-associated factor 1 (XAF1) has been identified to negatively regulate the caspase-inhibiting activity of XIAP. XAF1 mRNA is ubiquitously expressed in normal human tissue but is low or missing in majority of cancer cell lines. In this study, we explore the significance of XAF1 as a tumor suppressor in human hepatocellular carcinoma (HCC).

Methods: The expression of XAF1mRNA, the distribution of XAF1 protein in normal liver cell line L-02, hepatoma cell lines, SMMC7721 and HepG2, and 30 cases of hepatic carcinoma and their corresponding nontumor tissue specimens were examined by RT-PCR, immunohistochemical assay, and Western blot, respectively.

Results: XAF1 transcript and protein present at extremely low levels in both SMMC7721 and HepG2, Inversely, XAF1 transcript and protein can be detected in L-02. The expression of XAF1 mRNA and protein in HCC tissues was lower than those in the their corresponding nontumor tissue specimens (mRNA: 0.587±0.064，1.013±0.159，P <0.05; protein: 0.169±0.0280，0.643±0.0692，P <0.05). In additional, reduced XAF1 protein expression was correlated pathology grade.

Conclusion: XAF1 may play a key role in the hepatocarcinogenesis of hepatocellular carcinoma.

PP-075

MELD,CHILD score, and fibrosis indices in predicting outcome of acute variceal bleeding

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After control of the initial variceal hemorrhage, early rebleeding remains a risk.

Methods: Acute variceal bleeding was managed endoscopically in 965 patients (761 males, 204 females, mean age 52.6 years). All patients received antibiotic prophylaxis, and were followed for the occurrence of early rebleeding for 5 days if bleeding was controlled or till discharge.

Results: Mortality increased with increasing Child score: (0%, 2.4%, and 28% in Child A, B, and C, $p < 0.001$)) and MELD score (1.1%, 3.7%, 10%, and 48% in MELD ≤11; 12-15; 16-19; and ≥20 $p < 0.001$)). A cutoff of 20 for MELD had a sensitivity of 81.5% and specificity of 80.7% for predicting mortality and a cutoff of 10 for Child score had 80% sensitivity and 81.4% specificity. MELD and Child scores and the presence of encephalopathy had the highest power for predicting outcome (AUC= 0.889, 0.891, 0.886 respectively p<0.001). AST/ALT ratio, age-platelet index, APRI index, Phol's score, CDS and Goteborg University index ratio had lower discriminative power than MELD and CTP scores. Similarly, rebleeding increased with increasing Child and MELD scores. A shorter time between admission and endoscopy was a significant factor determining better survival and less rebleeding in patients with high Child and Meld scores.

Conclusions: Increasing MELD and CTP scores and the presence of encephalopathy are associated with high mortality in acute variceal bleeding. Urgent endoscopy remains a very important therapeutic intervention and should be offered earlier with patients with higher scores

PP-076

Declining incidence and mortality of hepatocellular carcinoma in Shanghai, China

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Objective: To characterize the temporal trends of the hepatocellular carcinoma (HCC) incidence and mortality rates in Shanghai, China.

Design: Retrospective cohort study.

Setting: Information on HCC incidence and mortality rates was collected from Shanghai population-based cancer registries system that is executed by Shanghai Center for Diseases Control.

Patients: Persons given a diagnosis of hepatocellular carcinoma between 1973 and 2004.

Methods: The time trends of the male and female incidence and mortality rates in the urban population from 1972 to 2004 were assessed using APC (annual percent change) analysis. The age-standardized incidence and mortality rates were calculated. The trends from 1972 to 2004 were tested using joinpoint regression analysis.

Results: From 1973 to 2004, the overall age-adjusted incidence rates of HCC deceased from the 37.07 per100 000 to 22.27 per 100 000 in male, from 11.72 per 100 000 to 7.09 per 100 000 in female. The APC of male and female age-standardized incidence continued to decrease during the past 32 years (-1.49% vs -1.68%, p<0.01). The median ages of incident cases in 2002 to 2004 were 60.65 years in male and 70.05 years in female. The overall age-adjusted mortality rates decreased from 32.43 per 100 000 to 18.16 per 100 000 in male, and from 11.78 per 100 000 to 5.66 per 100 000 in female. The APC of male and female age-standardized mortality continued to decrease during the past 32 years (-1.84% vs -2.12%, p<0.01).

Conclusions: Overall incidence and mortality rates of HCC have declined in Shanghai, China from 1973 to 2004，especially in male population.

PP-077

Analysis of side population in human hepatocellular carcinoma cell line MHCC97-H/L with different metastatic potential

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Objective: To identify the side population（SP）cells in hepatocellular carcinoma (HCC) cell line MHCC97-H/L and its tumor initiation.

Methods: MHCC97-H and MHCC97-L cell suspension were stained with Hoechst33342 and PI in the absence or presence of verapami, then sorted by Flow cytometer. To study the abilities of tumor initiation, the SP cells and non-SP cells were injected into the subcutaneous of the male non-obese diabetic/severe combined immunodeficiency（NOD/SCID）mice. MHCC97-H and MHCC97-L cells6) were examined by fluorescence microscopy after Hoechst33342 and carboxyfluorescein diacetate succinimidyl ester（CFSE）staining.

Results: The percentage of side population was 4.88% and 0.88% in MHCC97-H and MHCC97-L respectively, and decreased to 0.02% and 0.33% when cultured with verapamil. 5×104 SP cells were sufficient to tumor formation, while 1×106 non-SP cells did not initiate tumors. The percentage of side population was 4% and 1% in MHCC97-H and MHCC97-L respectively which showed by Hoechst33342 and CFSE staining.

Conclusion: MHCC97-H and MHCC97-L, which have different metastatic potential, all contain SP cells. The percentage of SP cell in MHCC97-H, HCC cell line with high metastatic potential was significantly higher than MHCC97-L, HCC cell line with low metastatic potential. SP cells can initiate tumors more effectively and maybe related to the metastatic potential of HCC.

PP-078

pERK is Associated With Microthrombus in Hepatocellular Carcinoma

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pERK is a biomarker of hepatocellular carcinoma (HCC) to the response to Sorafenib, and Raf/MEK/ERK pathway has a role in HCC This study aimed to determine whether pERK is associated with angiogenesis or microthrombi in peritumor tissue in HCC.

Methods: 120 patients underwent resection of HCC, and samples of the tumors were immunostained with pERK antibody to evaluate the relationship between cancer cell expression and microthrombi. Double immunohistochemical labeling for CD34 and pERK-positive cells was performed and classified as grade 0, grade 1, or grade 2 according to the number of double-positive cells. We also evaluated the relationship between the double-positive cell (endothelial) grading and microthrombi or MVD, and furthermore the relationship between cancer cell pERK immunoreactivity and MVD.

Results: Univariate analysis showed that patients with a median MVD exceeding 50/HPF had a significantly more microthrombi (p=0.02). The $pERK^{(+)}$ endothelial of grade 2 patients was significantly more microthrombi than that of the other two groups (p=0.011, p=0.0001), and the microthrombi of grade 1 patients has significantly more microthrombi than that of grade 0 patients (p=0.007). MVD differed significantly among the three grades (p=0.0007, Kruskal-Wallis test), and there was a significant positive correlation between MVD and grade (p=0.0001). No correlation was observed between MVD and the number of $pERK^{(+)}$ cancer cells alone (p=0.42). Neither the microthrombi nor MVD of $pERK^{(+)}$ patients differed significantly from that of $pERK^{(-)}$ patients (p=0.08, p=0.6).

Conclusions: $pERK^{(+)}$ endothelial might contribute to microthrombi, and thus may be an independent predictor to recurrence or metastases.

PP-079

The efficacy and safety of FOLFOX4 regimen in the treatment of locally advanced or metastatic hepatocellular carcinoma

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Background: Patients with locally advanced or metastatic hepatocellular carcinoma (HCC) have a poor prognosis and there is no effective and standard systemic chemotherapy regimen for them. Our objective is to investigate the clinical efficacy and safety of FOLFOX4 as palliative treatment for these patients who are unsuitable for surgery or localized therapy.

Methods and Results: Between April 2007 and February 2008, we used oxaliplatin with 5FU and leucovorin (FOLFOX4) every 2 weeks to treat 14 advanced HCC patients. There were 8 males and 6 females, and median age was 48 (range 31-74). Six patients were relapsed from prior surgery, while 8 were unresectable or ineligible to receive local invasive treatment at diagnosis. Six patients had cancer embolus in portal vein, nine patients had lung metastasis. AFP level was elevated in 12 patients. The median number of cycles delivered was 3.2 (1-13), and the total numbers were 45 with dose intensity of 91.4%. Among these patients, nine were eligible for response evaluation. According to RECIST criteria, there were 3 PR (33.3%), 4 SD (44.4%), but no patient achieved CR. The survival rate at three months was 78.3%（95% CI 47.3-92.5）, six month was 47.1%（95% CI 12.5-76.2）.AFP level was significantly decreased in four patients. The most common adverse events were: fatigue (6 patients), Ⅲ/Ⅳ myelosuppression (3 patients),peripheral neuropathy 2 patients),nausea and vomiting(2 patients),liver function impairment (2 patients).

Conclusion: FOLFOX4 regimen is an effective and well tolerated regimen for advanced hepatocellular carcinoma patients.

PP-080

Molecular mechanisms of hepatocarcinogenesis in DNp73-Transgenic mice: significance of N-Terminally truncated p73 Species for liver cancer therapy

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p53 family proteins carry on a wide spectrum of biological functions from differentiation, cell cycle arrest, apoptosis, and chemosensitivity of tumors. Conversely, N-terminally truncated p73 (deltaNp73) functions as a potent inhibitor of all these tumor suppressor properties, implicating its participation in malignant transformation and oncogenesis. Several reports indicated considerable upregulation of deltaNp73 in hepatocellular carcinoma (HCC) that correlates with reduced survival of patients, but little is known about the functional significance of deltaNp73 to tumorigenesis in vivo due to the lack of an appropriate model. To address this, we generated transgenic mice in which deltaNp73 expression is directed to the liver by the albumin promoter. Gene expression was tested by mRNA and protein analyses. Transgenic mice exhibited prominent hepatic histological abnormalities including increased hepatocyte proliferation resulting in preneoplastic lesions (liver cell adenomas) at 3-4 months. Among 12-to-20-months old mice, 83% of animals developed hepatic carcinoma. HCC displayed a significant increase of hyperphosphorylated inactive Rb, whereas p53-regulated inhibitors of cell cycle progression were downregulated in the tumors. Our data firmly establish the unique oncogenic capability of deltaNp73 to drive hepatocarcinogenesis in vivo, supporting its significance as a marker for disease severity in patients and as target for cancer prevention. This model offers new opportunities to further delineate deltaNp73-mediated liver oncogenesis but may also enable the development of more effective cancer therapies. Research was supported by grant 10-1884-St 1 of the Deutsche Krebshilfe to B.M. Pützer.

PP-081

Generation of transgenic mice with liver-specific expression of human genetic imprinting gene PEG10

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Paternally expressed gene 10 (*PEG10*) was a newly found genetic imprinting gene, which was highly expressed in a great majority of hepatocellular carcinomas, although its expression was absent in normal liver cells. We have found that the over-expression of PEG10 can inhibit cell apoptosis and the cell cycle, which was related to the decreased Cyclin D1 and increased p27 expression in HCC. The results

indicate that PEG10 participates in occurrence and development of HCC.

Objective: To establish transgenic mouse model that specifically expressed human PEG10 in the liver to facilitate the functional study of PEG10.

Methods: PEG10 cDNA was placed downstream of mouse albumin gene promoter to construct a liver-specific PEG10 expression vector. The linearized transgene fragments were microinjected into fertilized eggs of mice. The manipulated embryos were transferred into the oviducts of pseudo- pregnant female mice. The transcriptional specificity of peg10 was detected by reverse transcription polymerase chain reaction (RT-PCR).

Results: The linearized constructs were microinjected into 3741 fertilized eggs，and then the microinjected eggs were implanted into the oviducts of 94 pseudo-pregnant mice．In the 108 offspring, there were 8 mice carrying the transgene identified by polymerase chain reaction (PCR)．PEG10 were detected in the livers of the Alb-PEG10 transgenic mice using RT-PCR.

Conclusions: ALB-PEG10 fragment can integrate into mice genomic DNA. A hepatocyte-specific PEG10 transgenic mouse was generated by prokaryotic injection successfully which is useful for studying the function of PEG10 in vivo and discuss its significance in the hepatocellular carcino-genesis.

PP-082

Specific siRNA blocks anti-apoptosis function of HBV X and inhibits growth of hepatocellular carcinoma cells by silencing HBV X gene

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Objective: To investigate the effect of specific siRNA on HBV X expression, growth and apoptosis of hepatocellular carcinoma cells.

Methods: Two of small interfering RNA (siRNA) against adr and adw subtype HBV X mRNA were synthesized. HepG2/GFP-HBx cells expressing GFP-HBx fusion protein, and PLC/PRF/5 cells were transfected with the siRNA. Levels of HBV X mRNA in transfected cells were detected by semi-quantitative RT-PCR, and cell growth were evaluated by MTT method. HepG2/GFP-HBx and PLC/PRF/5 cells transfected with siRNA were treated with adriamycin (2.5 μg/ml), and apoptotic cell death were detected by TUNEL method.

Results: Levels of HBV X mRNA in specific siRNA-transfected $HepG_2$/GFP-HBx and PLC/PRF/5 cells were lower than that of non-treated cells and negative siRNA-treated cells. Growth rate of specific siRNA-treated cells were lower than that in non-treated cells and negative siRNA-treated cells ($P<0.05$). At 36 hours after treatment with adriamycin, apoptosis rates in specific siRNA-transfected HepG2/GFP-HBx (59.3%) and PLC/RF/5 (62.4%) were significant higher than that in non-transfected HepG2/GFP-HBx (3.5%) and non-transfected PLC/RF/5 (4.2%)($P<0.01$) while no different apoptosis rates were observed between $HepG_2$/GFP-HBx treated with specific siRNA, HepG2/GFP(61.3%) and HepG2 cells(57.8%) ($P>0.05$). However, no changed HBV X mRNA and cell growth and apoptosis rate were observed in cells transfected with negative siRNA.

Conclusions: The siRNA against HBV X is capable to inhibiting HBV X expression and anti-apoptosis function of HBV X, and inhibiting growth of HBV-related hepatocellular carcinoma cells. These suggest that siRNA may be a potential therapeutic agent for HBV-related HCC.

PP-083

Transfer of hepatitis C virus (HCV)-reactive T cell specificity for the treatment of HCV-associated hepatocellular carcinoma

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A gene therapy approach where retroviral vectors encoding the TCR genes from tumor or virus reactive T cell clones has been used to transfer the anti-tumor or anti-viral reactivity to normal PBL-derived T cells. The therapy in which T cells have been genetically modified with TCR genes to recognize HCV would represent a novel approach for the treatment of HCV infections and HCV-related malignancies. We have cloned and expressed two TCRs which mediate recognition of the 1406-1415 and 1073-1081 epitopes from the HCV NS3 protein. The results indicate that these TCR transduced T cells can recognize the wild type epitope, as well naturally occurring mutant variants of these epitopes. Most importantly, the TCR transduced T cells could also recognize HCV+ hepatocellular carcinoma cells. These data suggest this high affinity HCV-specific TCR might have potential new immunotherapeutic implications.

PP-084

Decreased TIP30 expression predicts poor prognosis in hepatocellular carcinoma

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Objectives: This study aimed to elucidate the role of TIP30 in the progression, early tumor recurrence (ETR), and prognosis of hepatocellular carcinoma (HCC).

Methods: Surgical specimens from 93 patients with HCC and its adjacent no tumor tissues were investigated by immunohistochemistry (IHC) for TIP30 and P53 using a standard detection system. The correlation of p53 mutation, clinicopathological factors and prognosis were analyzed.

Results: By IHC, undetectable or significantly decreased TIP30 expression was found in 59/93 (63.4%) of HCC compared with the adjacent no tumor tissues, mutated-type p53 immunopositive (mt-p53+) was detected in 38/93(40.8%) of HCC and none in all adjacent tissues. In 59 HCCs with decreased TIP30 expression, ETR and 2-year survival was 60.7% and 53.6%, respectively, vs. 5.9% and 91.2% in 34 HCCs with TIP30 overexpression. The HCCs which decreased TIP30 expression associated with mt-p53(+) had the poorest prognosis than other three groups.

Conclusion: TIP30 may play an important role in the suppression of carcinogenesis and progress of HCC. Assessment of TIP30 expression associated with p53 mutation in HCC may be used as a diagnosis marker for ERT and prognosis.

PP-085

A case-control study comparing percutaneous radiofrequency ablation alone or combined with transcatheter arterial chemoembolization for hepatocellular carcinoma

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Purpose: To assess whether combining percutaneous radiofrequency ablation (PRFA) with transcatheter arterial chemoembolization (TACE) was better than PRFA alone in patients with hepatocellular carcinoma (HCC).

Materials and Methods: 120 patients with one (≤ 7cm) to three (each δ3cm) HCC tumors were treated with PRFA combined with TACE (total 178 tumors). The results were compared with 120 patients with 163 tumors who were selected from a pool of 652 patients who received PRFA alone for HCC during the study period. These patients met all the selection criteria for the PRFA-TACE group of patients. The cases were matched at a 1:1 ratio between the two groups as far as possible in the following order of matching: size of lesion, site of lesion, number of lesions, age and sex. The survival outcomes of the PRFA group were not known at the time of matching.

Results: The 1-, 2-, 3-, 5-year overall survival rates for the TACE-PRFA and PRFA groups were 93.4%, 83.4%, 75.4%, 49.7%, and 88.5%, 75.6%, 63.6%, 42.3%, respectively($p=0.045$). On subgroup analyses, the survival for the TACE-PRFA group was better than the PRFA group for tumors >5.0cm ($p=0.031$) and for multiple tumors ($p=0.032$), but not for tumors ≤ 5.0cm ($p=0.319$) and solitary tumor ($p=0.128$). The 1-, 2-, 3-, 5-year progression free survival (PFS) for the TACE-PRFA and PRFA groups were 89.8%, 76.2%, 63.4%, 41.7%, and 76.3%, 59.5%, 47.0%, 30.2%, respectively ($p=0.002$). The number of tumors, serum alpha fetoprotein, serum albumin and the safety margin of ablation were significant prognostic factors.

Conclusion: Patients with HCC treated with TACE-PRFA had better PFS and overall survivals than PRFA alone.

PP-086

P H Domain Leucine-rich repeat protein phosphatase 1 is a novel target in hepatocelluar carcinoma therapy

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Protein phosphatases, together with protein kinases, control the balance of critical phosphorylation signaling events in cells. A recently characterized protein phosphatase, PH Domain Leucine-rich Repeat Protein Phosphatase 1 (PHLPP1), exhibited tumor suppressor activity in glioblastoma through attenuating AKT signaling. In this study, we investigated the role of PHLPP1 in heptocellular carcinoma, one of the most common cancer types in Asian population that are frequently accompanied with deregulated AKT signaling. PHLPP1 protein expression was much lower in the 7703 cancer cell line than in the normal counterpart cell line 7701. Immunohistochemistry studies using a panel of tumor and corresponding normal hepatic tissues further confirmed that PHLPP1 expression was significantly decreased in tumor. Overexpression of PHLPP1 in tumor cells resulted in marked reduction of cell growth, increased apoptosis and decreased migration, however, it exerted little effect in normal liver cells. In addition, mechanistic study suggested that pathways other than PI3K/AKT were involved in growth inhibition by PHLPP1. These results argue for an important role of PHLPP1 in liver tumorigenesis and suggest that PHLPP1 might be a valuable novel target for liver cancer therapy.

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PP-087

Experimental therapy for hepatocellular carcinoma by adenoviral-ribozyme mediated inhibition of survivin expression

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Reactivation of survivin expression is involved in the pathogeneses of solid tumors including liver cancer. Previous studies showed that inhibition of survivin promotes mitotic catastrophes and apoptosis of liver cancer cell in vitro. To explore the therapeutic application of survivin-targeted cancer therapy, we conducted the adenoviral ribozyme-based inhibition of survivin on hepatocellular cancer. Four ribozymes targeting the exposed regions of the SURVIVIN mRNA were cloned into vector pGVaL: R1 (the target nucleotide: +61), R2 (+83), R3 (+232) and R4 (+358), respectively, and then carried out in a non-replicative adenoviral vectors to generate catalytically active ribozymes. Cell proliferation, apoptosis, and tumor growth were assayed in vitro and in vivo. Infection of hepatocellular cancer cells with adenoviral-ribozymes dramatically suppressed the accumulation of survivin with the greatest suppression seen in R3. Combinatorial expression of R1, R3 and R4 together produced a synergistic suppression of survivin than any single ribozyme as the combination targets all three survivin transcripts. Furthermore, the ribozyme-mediated suppression of survivin induced mitotic catastrophe and hepatocellular cancer cell death via caspase 3-dependent pathway. More importantly, administration of the ribozyme prevents tumor formation and growth in a hepatocellular cancer xenograft model in vivo.These studies show that a combined suppression of survivin protein expression by ribozyme induced hepatocellular cancer cell death in vitro and inhibited tumor growth in animal. The adenoviral ribozyme system described in this report offers a potential robust gene therapeutic strategy in liver cancer treatment.

PP-088

Preventive effects of saikosaponin-d on experimental hepatocarcinogenesis in rats

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Objective: To study the preventive effects of saikosaponin-d on experimental hepatocarcinogenesis in rats.

Methods: Ninty SD male rats were randomly divided into 5 groups: normal control group（10）, hepatocarcinogenesis model group (20 induced by low doses of diethylnitrosamine interruptedly), saikosaponin-d group with different doses (2.0、1.5、1.0 ㎎/㎏).Rats were administerd with saikosaponin-d when they were induced by diethylnitrosamine for 14 weeks. All rats were killed in the 18th week, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT) and alpha-L-fucosidase (AFU) in serum were measured by biochemical examinations, at the same time, Hematoxy and eosin (HE) were used for examine the changes of liver pathology.

Results: The levels of ALT, AST, ALP, GGT, AFU in serum were significantly increased in model group in compared with those in control group, while the treatment with SSd markedly reduced all the above criteria compared with the model group, especial for the High dose group. (162.56±20.70 vs 410.01±93.62, 210.45±22.76 vs 400.25±90.02, 220.62±24.65 vs 410.45±94.85, 75.45±24.02 vs 157.84±6.75, 126.63±25.13vs200.16±7.31 ， $P < 0.05$).Similarly, histological examination demonstrated that SSd could attenuate the grade of cancer cell differentiation.

Conclusion: Saikosaponin-d have certain preventive effects on experimental hepatocarcinogenesis in rats.

PP-089

HCRP1, a putative tumor suppressor gene, is the important regulator of EGF-induced down-regulation of epidermal growth factor receptor

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Background: Overexpression of EGFR is implicated in the initiation and development of hepatocellular carcinoma. However, mechanisms of EGFR overexpression remain elusive and often can be attributed to gene amplification. HCRP1, a novel gene isolated by our lab, was localized at chromosome 8p22 on which high LOH frequency was detected in HCC.

Methods: We used small interfering RNA to knock-down the expression of HCRP1 in HCC cells (Bel-7404 and SMMC-7721), normal liver cell QSG7701 and mouse fibroblast cell NIH3T3. Besides, we utilized western blot analysis and fluorescence microscopy to investigate the role of HCRP1 in EGF-induced EGF receptor down-regulation. Moreover, pulse-chase experiment was conducted to analyze its function in regulation of downstream signaling molecules ERK and STAT3 activity after EGF treatment.

Results: Using siRNA, we established the corresponding HCRP1 knock-down stable cell lines. Further experiments showed that HCRP1 knock-down delayed EGF-induced EGF receptor degradation in these cell lines and promoted the extent and magnitude of downstream signaling molecules ERK and STAT3 activity when treated with EGF.

Conclusions: Our studies suggested an important role of HCRP1 in endocytosis-mediated EGF Receptor degradation and EGF-coupled signaling pathway, and provided a new clue for EGFR deregulation in HCC.

PP-090

The effect of mesenchymal stem cells on in vivo proliferation and in vitro metastasis of hepatocellular carcinoma

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Aim: To explore the possible role of mesenchymal stem cell (MSC) in the progression of HCC and the mechanism involved.

Methods: The MHCC97-H cells (with a high metastatic potential) were co-cultured with MSC-conditioned medium (MSC-CM). The nude mice models of HCC were applied to evaluate the *in vivo* effects of MSC on HCC. The MHCC97-H cells tansfected with a GFP expression plasmid vector, and MSC labeled with DAPI were used to detect the *in vivo* distribution of MSC. The expression levels of related markers in HCC cells and tumor tissues were analyzed by real-time PCR and Western blot.

Results: Co-culturing with MSC-CM could significantly promote the proliferation of MHCC97-H cells. The MSC was found to be merged into tumor tissues. As compared with the controls, both the tumor size and the median tumor/body weight ratio in the experiment mice were larger (3080.51±1234.78 vs. 2223.75±1000.60 mm^3, p=0.046; and 0.15 vs. 0.13, p=0.03, respectively). However, the incidence and the cellular numbers of lung metastasis were significantly decreased (50 % vs. 100 %, p=0.015; 49.75±53.35 vs. 227.22±224.00, p=0.046, respectively). The alterations in the expressions of TGF-β1, MMP2, Smad2 and smad7 were found to correlate with tumor growth and metastasis. Moreover, close correlation between the expressions of TGFβ1 and MMP2 was also found.

Conclusions: MSC could enhance the HCC growth, but significantly inhibit the pulmonary metastasis of HCC, which might be induced by

the abnormal expression of TGFβ1 and down-regulation of TGFβ1/Smad pathway. These suggest that transfusion of MSC might be a novel strategy in the control of metastatic recurrence of HCC after operation.

PP-091

Therapeutic effect of adenovirus and alpha-fetoprotein-mediated tBid on orthotopic hepatocellular carcinoma (HCC) tumor in mice

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Bid plays a role in the development of HCC and the application of alpha-fetoprotein (AFP) promoter-mediated tBid (Ad/AFPtBid) significantly and specifically kills AFP-producing Hep3B cells *in vitro* and in mice subcutaneously implanted with HCC cells. This study further tested the Ad/AFPtBid in an orthotopic hepatic tumor model, which represent naturally occurring HCC. In this model, Hep3B cells were injected into the liver of nude mice. Four weeks after implantation, the mice were randomized into 3 groups. One group was treated with $1x10^{10}$ pfu of virus via tail vein injection every 2 days for totally 3 times, and the other two groups were treated with PBS and Ad/AFPLacZ as controls. AFP in serum was determined by ELISA weekly to monitor the tumor progression. The mice were killed four weeks after treatment. The size of the liver tumor was significantly reduced in the mice treated with Ad/AFPtBid, compared with the other two control groups, indicating that Ad/AFPtBid treatment significantly inhibited Hep3B tumor growth. Tumor tissues of Ad/AFPtBid-treated mice showed a decrease in cells positive for PCNA that is a marker of cell proliferation. Lymphocyte infiltration was increased in the tumor tissues of Ad/AFPtBid-treated mice, which may be an indicator of occurrence of tBid-mediated cell damages. In conclusion, Ad/AFPtBid can specifically target the APF-producing HCC in an orthotopic mouse model of HCC and thus it supports the development of Ad/AFPtBid as a gene therapy for HCC. (This work was supported by the Research Grants Council of the Hong Kong SAR. No: CUHK4534/06M).

PP-092

Tumor metastasis suppressor HTPAP's haplotype reconstruction in plasma circulating DNA and their relations to invasion potential of hepatocellular carcinoma

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Objective: In our previous studies, HTPAP was found to be a candidate metastasis suppressor gene for hepatocellular carcinoma (HCC). In this study, we want to analyze the haplotypes of HTPAP through identifying single nucleotide polymorphisms (SNPs) in plasma circulating DNA, and their relations to invasion ability of HCC.

Methods: A total of 20 SNPs mapped at HTPAP were detected in the plasma circulating DNA from 80 HCC cases. The 74 cases were enrolled to haplotype reconstruction by PHASE software.

Results: Among 20 SNPs, 13 were monomorphic, 2 yielded no significant results, and the remaining 5 SNPs were enrolled in the haplotype analysis. A total of 11 haplotypes were found, and 4 of them (C-A-T-C-A,T-G-G-G-G,C-G-T-C-A,T-A-G-G-G) occurred most frequently (90.5%), and accounted for 64 cases(86.5%). Four SNPs （rs3739252A/G、rs1149C/G、rs3830326－/TAAG、rs7007097C/T）were found a strong linkage disequilibrium, the other one at rs11539529 had a historical recombination, however, all of them were considered to belong to one haplotype block. Association analysis revealed that carriers of alleles rs3739252G, rs1149G, rs3830326TAAG, rs7007097T and their related haplotype T-G-G-G-G, T-A-G-G-G were significantly associated with high metastatic potential of HCC ($P< 0.05$).

Conclusions: Four common haplotypes (C-A-T-C-A,T-G-G-G-G,C-G-T-C-A,T-A-G-G-G) were found in these Chinese HCC patients, and the functional analysis of different genotypes and haplotypes of HTPAP might provide an new way in the prediction of patients' prognosis and tumor recurrence.

PP-093

Cloning and characterization of LI-cadherin (CDH17) gene promoter in human HCC

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The Liver Intestine-cadherin (LI-cadherin, CDH17) is a structurally divergent member of the cadherin superfamily. Over-expressed LI-cadherin was found in human gastric cancer and hepatocellular carcinoma (HCC). Intrinsically, the over-expression of LI-cadherin may be due to chromosomal gains of 8q. However, other extrinsic causes such as gene regulation may also contribute to its high expression in HCC. To further investigate the gene regulatory mechanism of CDH17, the 5-flanking region was cloned. Deletion constructs were transient transfection into HCC cell lines and promoter activity of CDH17 was then determined to be dependent on positive regulatory elements located within 100 nucleotides directly upstream of the transcriptional start site. Within this minimal promoter region, several potential transcription factors were explored to be caudal related homeobox 2 (CDX2), hepatic nuclear factor 1 (HNF1) as two enhancers and CAAT displacement protein (CDP), B cell lymphoma 6 (BCL6) as two repressors. Then site-directed mutagenesis was performed and Electrophoretic Mobility Shift Assay (EMSA) and Chromatin Immunoprecipitation (ChIP) were also used to verify the mutual interaction in vitro and in vivo. Interestingly, we found there was significant difference in luciferase activity between two cell lines we chose for transfection. After comparing the different protein expression of these four potential transcription factors and LI-cadherin in cells as well as mouse liver tissue, we found the trend of LI-cadherin expression was correlated well with those of enhancers and repressors. From above we concluded that the difference may be mainly due to various enhancers and repressors expression as well as LI-cadherin expression.

PP-094

CIDE-B, a gene with different expression levels between normal liver tissues and liver cancer samples induces apoptosis through mitochondrial pathway

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Background & Aims: Identification genes with different expression levels between normal liver tissues and liver cancer samples may provide potential targets for the therapy of liver cancer. Cell death-inducing DFF45-like effector B (CIDE-B) is one of these genes. CIDEs induce apoptosis in mammalian cells. We have studied the mechanisms of CIDE-B-induced apoptosis and the regulation of tissue- and cell- specific expression of CIDE-B to discover the relationship between CIDE-B and liver cancer development.

Methods: We confirmed expressed sequence tags (EST) between normal liver tissues and liver cancer samples by RT-PCR and Northern blot analysis. Apoptosis was detected by DNA fragmentation analysis and propidium iodide staining and flow cytometry.

Results: CIDE-B was expressed highly in normal liver tissues and lowly in liver cancer samples. CIDE-B had two promoters: the upstream and the internal promoter. The upstream promoter was hypermethylated in cells that did not express the long transcript of CIDE-B, but was hypomethylated in cells that expressed this transcript. Overexpression of CIDE-B in CHO, 293T and BEL-7404 cells induced apoptosis. Transfection of GFP-tagged CIDE-B into 293T and BEL-7404 cells revealed that CIDE-B displayed partial overlapping with mitochondria but no overlapping with peroxisomes-, Golgi-, endoplasmic reticulum- or lysosomes-specific markers. CIDE-B induced cytochrome c releasing from mitochondria and activated caspase-3 in 293T and BEL-7404 cells as demonstrated by immunofluorescence.

Conclusions: The expression of CIDE-B is tissue- and cell- specific. CIDE-B induces apoptosis in 293T and BEL-7404 cells through mitochondrial pathway.

PP-095

Endothelial cells enumeration in peripheral blood of patients underwent hepatectomy – Initial results

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Purpose: To investigate the circulating endothelial cells (CEC) and endothelial progenitor cells (EPC) counts in peripheral blood of patients who underwent hepatectomy and their variation a week after operation.

Methods: Forty-nine patients underwent hepatectomy were recruited in this study, including hepatocellular carcinoma (HCC, n = 31), intrahepatic cholangiocellular carcinoma (n = 3), benign disease (n = 9) and metastatic liver cancer (n = 6). CEC and EPC in their pre-operative and a week post-operative peripheral blood were enumerated by using four-color FACS. Quantification of CEC in 100uL blood using CD45-, CD31+, and CD34+ as markers, and CD133+ was used additionally to identify EPC.

Results: Patients with malignant disease had lower relative CEC numbers (total CEC/total granulocytes) than with benign disease (2.8% vs 4.1%, p =0.019). Patients with HCC had lower post-operative EPC numbers than pre-operative counts, but the significance was borderline (0.05‰ vs 0.11‰, p =0.092). The post-operative relative CEC counts in patients with HCC positively correlated with tumor diameters (r = 0.645, p = 0.007).
Conclusion: CEC counts showed some clinical significance between pre-operation and post-operation, and between patients with malignant and benign diseases, which need to be further validated in the larger cohorts of patients.

PP-096
Nucleophosmin/B23 targets p21WAF1/CIP1 and contributes to cell cycle arrest of HepG2 cells induced by actinomycin D
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Nucleophosmin (NPM)/B23, a nucleolar phosphoprotein, has been shown to interact with many proteins like p53, ARF, and Rb. Here we showed that Actinomycin D (ActD) caused NPM nucleoplasmic redistribution and G2 arrest in HepG2 cells and enhanced interaction between NPM and p21. By utilizing analyses of endogenous and ectopically expressed proteins, we demonstrated that NPM bound directly to p21 *in vivo* (Fig. A and B) and *in vitro* through its C-terminal. NPM was shown to prolong p21's half life, which was interdicted by NPM siRNA (Fig. C). Knock down of NPM induced increase of the cells in S phase and change from G2 phase arrest by ActD treatment to S phase arrest by NPM siRNA and the drug treatment (Fig. D).Given the overlapped functions between NPM and p21, we propose that NPM is a positive regulator of p21 involved in cell cycle progression.

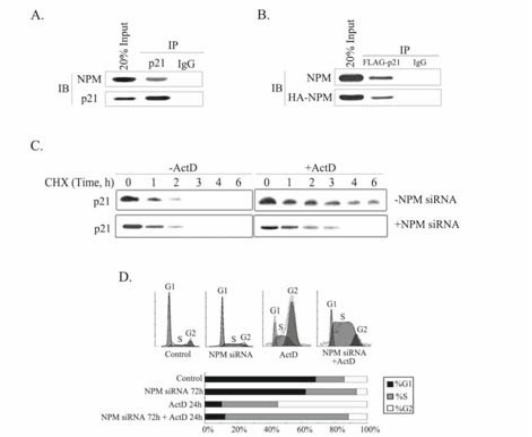

Fig. A and B, showing that NPM interacts with p21 *in vivo*.

Fig. C, showing that NPM prolongs half-life of p21.

Fig. D, showing that NPM siRNA induces increase of the cells in S phase and change from G2 phase arrest by ActD treatment to S phase arrest.

PP-097
A garlic derivative S-allylcysteine suppresses liver tumor growth and metastasis by sensitizing chemotherapy
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Background and Objective: A garlic derivative S-allylcysteine (SAC) has anti-cancer effect in human prostate and colon cancers. We aimed to investigate the effect of SAC and combination of chemo-drug on tumorigenesis and metastasis of liver cancer.
Materials and Methods: The orthotopic liver tumor model using a metastatic liver cancer cell line MHCC97L labeled with luciferase gene was applied. SAC was given at day 7 after tumor implantation at 1mg/g/day, 2mg/g/day, or 1mg/g/day combined with low dose Cisplatin for 5 weeks. Tumor growth and metastasis were monitored by Xenogen *in vivo* imaging system. Hepatic stellate cell (HSC) activation and tumor-associated macrophage (TAM) in the tumor tissue were detected by α-SMA and ED1/ED2 staining. Tumor micro-vessel density (MVD) and apoptosis were also analyzed. *In vitro* functional tests including MTT assay, colony formation assay, cell cycle analysis and apoptosis analysis were performed.
Results: The tumor growth was significantly inhibited by SAC combined with Cisplatin treatment at different time points accompanied by lower incidence of lung metastasis compared with other groups. The observation of Xenogen IVIS was confirmed by histopathological examination. The HSC activation by α-SMA staining in the liver tumors was suppressed by SAC and Cisplatin treatment accompanied with less TAM infiltration. Consistent with in vivo study, in vitro functional study also demonstrated that SAC not only induced cell cycle arrest and tumor cell apoptosis, but also significantly sensitized the anti-cancer effect of Cisplatin.
Conclusion: SAC treatment significantly inhibited liver tumor growth and metastasis by induction of tumor cell apoptosis and together with sensitization of chemotherapy.

PP-098
Identification and characterization of hepaCAM, a novel immunoglobulin-like cell adhesion molecule that promotes cell-matrix interactions and exhibits growth inhibitory effect on liver cancer cells
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Genetic alterations have been defined as the hallmark of cancers as they are responsible for the differences between normal and malignant phenotypes. In this study, using the technique of suppression subtractive hybridization, we examined differential gene expression in hepatocellular carcinoma (HCC) and identified a new member of the immunoglobulin superfamily, "hepaCAM". RT-PCR showed hepaCAM expression was significantly decreased in 20/23 of HCC specimens and undetectable in 5/6 HCC cell lines. Mapped to chromosome 11q24, hepaCAM had no significant similarities to any known genes. Its protein of 416 amino acids displayed a typical structure of Ig-like adhesion molecules, including two extracellular Ig-like domains, a transmembrane segment and a cytoplasmic tail. Through transfection studies on HepG2 cells, we explored the biochemical characteristics and biological functions of hepaCAM. The gene product, a 75 kDa protein, was glycosylated and phosphorylated, and formed *cis*-dimers on cell surface. The subcellular distribution of hepaCAM appeared to be cell density-dependent and colocalized with F-actin. Functionally, hepaCAM reduced cell colony formation (P = 0.0022), inhibited cell proliferation ($P < 0.001$) and arrested cells in the G2/M phase. In addition, hepaCAM increased cell spreading and cell migration. Interestingly, when the cytoplasmic domain was deleted, the mutant could less significantly increase cell-matrix adhesion, cell motility and growth inhibition compared to the wildtype. In conclusion, gene hepaCAM is frequently downregulated in HCC. The gene encodes a new membrane-associated glycoprotein, an Ig-like cell adhesion molecule, modulating cell-matrix adhesion, cell motility and cell growth. The cytoplasmic domain of hepaCAM is essential to its function.

PP-099
Alpha-fetoprotein induced phosphorylation of AKT via inhibited the activity of PTEN in human hepatoma Bel 7402 Cells
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Objective: Alpha-fetoprotein (AFP) has a property to maintain the growth of hepetocellular cancinoma cells (HCC) in vivo, but the critical functional step of AFP still obscurity, the present investigation in order to explore AFP influence on the signal transduction of PTEN-AKT in HCC.
Methods: Western Blotting was utilized to detecting the expression of PTEN when human hepatoma cells line Bel 7402 were treated with all *trans* retinoic acid (ATRA) for 24h; AFP interacted with PTEN was analyzed by co-immunoprecipitation (Co-IP); Laser confocal microscopy was used to observe co-localization of AFP and PTEN;

Short small RNA interfering (RNAi) technique was applied to knockdown the expression of AFP in Bel 7402 cells; Fluorescence resonance energy transfer (FRET) of FITC (labeled PTEN) and TRITC (labeled AFP) was analyzed by laser confocal microscopy and the phosphorylation of protein kinase B(AKT) was detected by Western blotting. Results: It showed that Bel 7402 cells expressed PTEN, and ATRA（160μmol/L）could promote the expression of PTEN mildly; Co-IP indicated that AFP has a property to interact with PTEN in cytoplasm, PTEN and AFP was observed colocalize in cytoplasm of Bel 7402 cells; It is also showed that FRET was generated between FITC and TRITC which labeled PTEN and AFP respectively, and the analysis of FRET found that the fluorescent molecules distance was 6.7±1.5 Å ; RNAi could knockdown the expression of AFP; The expression of PTEN was promoted and phosphorylation of AKT was decreased when RNAi the expression of AFP.
Conclusions: These data provide that AFP has a capability to interact with PTEN and inhibit the activity of PTEN, this is also the pivotal events that AFP activated the transduction of PI3K/AKT signal of hepatoma cells.

PP-100
Tumor microvessel density is associated with tumor perfusion measured by contrast-enhanced ultrasound in hepatocellular carcinoma
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Objective: To correlate contrast-enhanced ultrasound parameters with micro vessel density of hepatocellular carcinoma (HCC) and to find a reliable non-invasive method to evaluate tumor vascularity in vitro.
Methods: 2006 Nov to 2007 Mar, 27 patients with resectable HCC were observed with Contrast-enhanced ultrasound. Enhancement phases of tumor lesions were analyzed and compared with tumor-free liver tissues beside. Using immunohistochemistry, the slides of tumor specimens were stained by anti-CD34 mono-antibody. The areas of the positive staining was evaluated by Leica Qwin plus V3 software under the magnification of 50×, and the MVD was calculated as percentage of positive area/total area.
Result: Mean MVD of these patients was 1.95±1.18%. Spearman correlation test revealed that the MVD was correlate with CEUS parameters in SI(r=0.508 P= 0.007), AUC (r=0.651 P<0.001), and P (r=0.676 P< 0.001)
Conclusion: There's correlation between MVD and CEUS parameters (SI, AUC, P). These parameters can substitute MVD to evaluate the effect in anti-vascular therapy.

PP-101
Combination of sunitinib and trans-arterial embolization suppresses angiogenesis and growth of hepatocellular carcinoma in Buffalo rat
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Unresectable hepatocellular carcinoma (HCC), generally with hypervascularity, has been commonly treated with trans-arterial embolization (TAE), but shown high recurrence and metastasis in which angiogenesis played a critical role. Sunitinib, multi-targeted receptor tyrosine kinases inhibitor, blocks vascular endothelial growth factor receptor 2 (VEGFR-2), platelet-derived growth factor receptor β (PDGFR-β), fetal liver tyrosine kinase 3(FLT-3), etc., and has displayed the potent anti-angiogenetic activity in renal carcinoma. The study was conducted to evaluate anti-tumor efficacy with the combination of sunitinib and TAE in HCC. A panel of cell lines were applied to test the in vitro effects of sunitinib, including human HCC (HepG2, Hep3B), rat(McA-RH7777), human liver cell(L-02) and human umbilical vein endothelial cell(HUVEC). Sunitinib inhibited proliferation and induced apoptosis (by flow cytometry) of HCC at 1μM and HUVEC at 0.01μM significantly, which was correlated with inhibition of phosphorylation of VEGFR-2 and PDGFR-β (by immunoblotting and immunohistochemistry). Buffalo rat HCC models were treated with TAE or/and sunitinib (via intra-aretial, p.o. or intra-arterial before p.o.) and The initial enhancement slope (IS) by dynamic contrast-enhanced MRI (DCE-MRI) was used to analyze the in vivo anti-tumor efficacy. Combination of TAE and sunitinib exhibited substantial tumor growth arrest, increase of necrosis and decrease of tumor perfusion. Tumor apoptosis (by TUNEL), micro-vessel density (MVD, labeled by CD31) and inhibition of phosphorylation of VEGFR-2 and PDGFR-β were verified which indicated clear correlation with IS. Given these results, combination of sunitinib and TAE can suppress angiogenesis and inhibit the growth of HCC. Sunitinib demonstrates its potential in combination with TAE in the future.

PP-102
Suppression of HSF1 via RNA interference enhances the sensitivity of HepG2 to chemotherapeutic agents
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Background: HCC is highly resistant to currently available chemotherapeutic drugs. Heat shock factor 1 (HSF1) is the master regulator of the heat shock response in eukaryotes. Recently, HSF1 has been identified to have the opposite effect in supporting the lethal phenomenon of cancer. In this study, we investigate the influence of siRNA targeting HSF1 on the sensitivity of HepG2 to chemotherapeutic agents.
Methods: HSF1 siRNA and negative siRNA expression vector were constructed and transfected into HepG2 cells. RT-PCR, western blot were used to detect the target gene expression. Drug sensitivity of HepG2 to mitomycin C and 5-Fluorouracil was analyzed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay and flow cytometry.
Result: RNA interference efficiently down regulated HSF1 expression in HCC cells. MTT results showed that HSF1 siRNA transfected cells have a higher cell inhibition rate than negative vector transfected cells or untreated cells after treatment with 5-Fluorouracil (130-1300 mg/L) and mitomycin C (0-20 ⌈M). Flow cytometry results demonstrated that the sub-G1 population increased in the HSF1 siRNA group after treatment with 5-Fluorouracil (1300 mg/L) and mitomycin C (20 μM). We demonstrate that HSF1 knockdown significantly augmented apoptosis sensitivity of HCC cells towards chemotherapy.
Conclusion: Suppression of HSF1 via RNA interference is a promising approach to sensitize HCC cells towards chemotherapy.

PP-103
Mechanism of anti-angiogenesis by interferon alpha in human hepatocellular carcinoma cell with up-regulation of thymidine phosphorylase
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Background: Thymidine phosphorylase (TP) is the key enzyme for the chemotherapeutic of fluoropyrimidines. TP has also been shown to possess angiogenesis activity in vivo and chemotactic activity in vitro. Our previous studies indicated that interferon-alpha (IFN-alpha) enhanced the anti-tumor effect of capecitabine on hepatocellular carcinoma (HCC) by the up-regulation of TP expression in liver cancer tissues, and that certain dose of IFN-alpha up-regulated the TP expression, and inhibited the angiogenesis induced by TP in vivo and in vitro as well. We hypothesized that IFN-alpha down-regulates other genes expression related to pro-angiogenesis, the same as up-regulates TP gene expression.
Method: Human HCC cells of the line SMMC-7721 were cultured and added with IFN-alpha of different doses 0 (as control group) and 10000U/ml. Twenty-four hours later cDNA array was used to detect the gene expression related to angiogenesis. Real-time quantitative PCR was used to confirm the gene expression.
Results: Gene chip used contains 116 genes related to angiogenesis. In the dose of 10000U/ml IFN-alpha group, 14 genes expression levels were above two times as control group, such as TP, and granulocyte colony stimulating factor 3, but another 10 genes expression levels were lower two times, such as vascular endothelial growth factor C, interleukin 8, and chemokine ligand 11.
Conclusion: Brown et al (2000) reported that the TP-mediated generation of reducing sugars leads to oxidative stress and the subsequent release of angiogenic factors, such as VEGF, IL-8 and MMP-1, from tumor cells. IFN-alpha of certain doses up-regulates the TP expression, but inhibits the angiogenesis by down-regulation of other genes related to pro-angiogenesis as well.

PP-104
Diagnosis of hepatic lymphoma
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Objective: To investigate the value of diagnostic methods of primary hepatic lymphoma (PHL).
Methods: We reviewed retrospectively the clinical presentation and imaging findings in ten patients in whom a diagnosis of PHL was finally made histologically.
Results: The main clinical manifestations of PHL were right upper quadrant pain (five patients), hepatomegaly (four patients) and fever (two patients). Seven patients were hepatitis B surface antigen-seropositive. Six patients had preexisting liver diseases and four patients had chronic hepatitis or cirrhosis. None had peripheral lymphadenopathy. All foci of PHL were hypoechoic relative to normal liver on ultrasound (US) images except one patient. Computered tomography (CT) showed hypoattenuating lesions in all cases. Four patients were misdiagnosed as hemangioma or other benign lesions by radiological methods.
Conclusion: Clinical presentation and imagings of PHL are non-specfic so that histology is required in all patients to establish the diagnosis. Differential diagnosis is necessary when we detected hypoechoic lesions that shows atypical features like primary liver carcinoma or hemangioma on the sonogram. And the lesions that we considered as liver metastases when no primary tumor outside the liver was apparent should be distinguished from PHL.

PP-105
Induction of dendritic cells from intraoperative lost blood and their effects on cytokine induced killer cells against hepatocarcinoma in vitro
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Objective: To prepare mature dendritic cells (DCs) from mononuclear cells in lost blood during hepatectomy for hepatocellular carcinoma (HCC).
Methods: Mononuclear cells isolated from cord blood or intraopertive lost blood were induced with rhGM-CSF plus rhIL-4 and differentiated into immature DC, which were then loaded with lysate antigen of the human hepatocarcinoma cell line SMMC-7721 and autologous liver cancer cells from patients with HCC and the mature DCs were obtained. Both DC vaccines were identified by light microscope, electron microscope, FACS and immunohistochemistry. MTT assay was used for measuring their effects on T lymphocyte proliferation and their efficacy in mediating the cytotoxicity of cytokine induced killer cells (CIK) against liver cancer.
Results: Mononuclear cells separated from intraoperative lost blood or cord blood were induced into DCs possessed typical morphorlogy and phenotypes. Mixed lymphocyte reaction showed the average growth rates of CIK activated by DCs loaded with the patient's antigen, the SMMC-7721 antigen or without loading antigen are 388.9%; 239.9% and 134.3%, respectively and, their DC-mediated specific cytotoxicity against the cancer cells from patients were 87.1% 76.4%, 58.8%, respectively, while the control group without DCs was only 49.8%.
Conclusion: Mononuclear cells separated from intraoperative lost blood of the patients with HCC can be induced into the typical mature DCs, which can effectively activate CIK and exert increased killing to the liver cancer cells in vitro.

PP-106
Haplotype reconstruction for tumor metastasis suppressor HTPAP and their relations to invasion ability of hepatocellular carcinoma
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Objective: In our previous studies, HTPAP was found to be a candidate metastasis suppressor gene for hepatocellular carcinoma (HCC). In this study, we want to analyze the haplotypes of HTPAP through identifying single nucleotide polymorphisms (SNPs) on it, and their relations to invasion ability of HCC.
Methods: We detected SNPs by direct sequencing of 6.5kb including HTPAP (4.5kb) and its 5'-flanking region (2kb), and then analyzed genotypes of the SNPs in DNA samples from 200 cases of microdiessected fresh HCC tissues. The 200 cases were enrolled to haplotype reconstruction by PHASE software.
Results: Six SNPs were detected (-1053 A/G, -625 G/C,+357C/G, +1648-/TAAG,+1838A/G, +3528C/T). Five SNPs were found a strong linkage disequilibrium, the other one at +1838A/G had a historical recombination. A total of 11 haplotypes were found, four of them (C-A-T-C-G-A,T-G-G-G-C-G,C-G-T-C-G-A,T-A-G-G-C-G) occurred most frequently (94.75%),and accounted for 184 cases(92%). Association analysis revealed that carriers of alleles -1053G, -625C,+357G, +1648TAAG, +3528T and their related haplotype T-G-G-G-C-G, T-A-G-G-C-G were significantly associated with high metastatic potential of HCC ($P< 0.05$).
Conclusions: Four common haplotypes (C-A-T-C-G-A, T-G-G-G-C-G, C-G-T-C-G-A, T-A-G-G-C-G) were found in these Chinese HCC patients. And the functional analysis of different genotypes and haplotypes of HTPAP might provide an new way in the prediction of patients' prognosis and tumor recurrence.

PP-107
Protein kinase Cβ induces HCC cells motility and invasion in vitro
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Considerable interests have recently been focused on mechanism of HCC metastasis-the most fundamental characteristics of HCC and the ultimate cause of most HCC mortality, so screening more potential early prognostic marker and therapeutic target is urgent. In this study, we screened genome of three HCC cell lines with consistently increased metastatic potentials, which share same genetic background, through DNA microarray and found consecutively up-regulated expression of PKCβ in these cell lines, which was reconfirmed by real time RT-PCR and western blot analysis. Moreover, it was found, after efficient silence of PKCβ by RNAi assay or inhibition of PKCβ activity by a specific inhibitor LY317615, migration and invasion of HCC cells significantly decreased. In addition, depletion of PKCβ protein significantly reversed the enhancement of PMA-stimulated HCC migration and invasion ability *in vitro*. All the data suggest a key role of PKCβ in HCC metastasis and PKCβ may be a potential therapeutic target.

PP-108
Liver cancer: ephrin-A2 promotes tumorigenicity and metastasis through PI3K/Akt/NF-kappaB signaling pathway
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Ephrin-A2, a ligand of the EphA family of receptor tyrosine kinases, has been well studied in embryogenesis, morphogenesis and angiogenesis, while its function in tumor development and progression remains to be characterized. In this study, we find that the expression level of ephrinA2 is significantly upregulated in both liver cancer cell lines and primary hepatic carcinomas from liver cancer patients. Forced expression of EphrinA2 into liver cancer cells can effectively promote the tumor growth *in vivo*, which can be reversed by the siRNA targeting EphrinA2. In addition, the EphrinA2 transfectants are endowed with resistance to TNF-alpha induced apoptosis through PI3K/Akt/ NF-kappaB signaling pathway. Knockdown of EphrinA2 was able to elevate the sensitivity of EphrinA2 transfectants to TNF-alpha induced cell death. These findings identify a novel Ephrin ligand signaling pathway positively regulating tumorigenicity of hepatocarcinoma cells. EprinA2 may be a potential target of therapeutic intervention for inhibition of tumor progression in liver cancer.

PP-109
Studies on the features of loss of heterozygosity in early small hepatocellular carcinoma and precancerous lesions
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Objective: Up to know, we know a little about the genetic connection between hepatocellular adenomas (HCA) dysplastic nodules (DNs) with the precancerous nature and hepatocellular carcinomas (HCC). The present study is designed to identify genetic markers that could serve as a putative predictor for early progression of HCC, and is helpful for clinic to evaluate the postoperative prognosis.
Materials and Methods: Archival paraffin-embedded tissues including 10 HCA, 10 DNs and 49 HCC in our pathological department were chosen. The analysis of loss of heterozygosity (LOH) on 24 microsatellite loci detected by polymerase chain reaction-based microsatellite polymorphism using ABI3700 automatic DNA analysis system were carried out in both tumors and corresponding adjacent noncancerous liver tissues.
Results: Frequency of LOH on 4q, 9p, 17p in HCC was higher significantly than that in DN (0 vs 55.6%, 11.3% vs 33.5%, 18.0% vs

53.2%). Increasing fractional allelic loss (FAL) values were seen from DN to Micro HCC, and further to small HCC(0.106±0.139→0.377±0.198→0.493±0.276). FAL value was higher significantly in tumors with Edmondson's grade more than Ⅲ (P=0.035) and in cases with liver cirrhosis (P=0.031).

Conclusion: These results suggested that there are specific patterns of genetic instability common to DNs and the HCC that subsequently develop even when the paired lesions are not clonally related. It indicated that there was some common genetic pathways in the malignant transformation of normal cells. Our results favored a concept of accumulation of multiple genetic alterations contributing to the development and progression of HCC.

PP-110

Radiofrequency thermal ablation of small and medium sized hepatocellular carcinomas in cirrhotic patients

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Background: Most patients with HCC are not candidates for resection because of tumor location, multifocality, or severity of underlying cirrhosis. RFA is a technique that permits in situ tumor destruction by means of local tissue heating.

Methods: Thirty-one cirrhotic patients (Child's class A, 12, B, 19) with 47HCC nodules were treated with RF using either percutaneous or intraoperative approach under ultrasound (US) guidance to place the RF needle electrode into the hepatic tumors. Fifteen nodules were small (≤3 cm) and 32 were medium (3.2-4.8 cm). The mean diameter for all tumors was 3.1 cm. All patients were followed up at regular intervals to detect treatment-related complications or recurrence of disease.

Results: After a single RFA session, complete necrosis was achieved in 35 (74.5%) out of the 47 treated HCC nodules, partial or incomplete necrosis was achieved in 11 (23.4%) nodules, while the target temperature could not be reached in the remaining one HCC tumor (2.1%). Small tumors (≤3 cm) were treated successfully more often than medium (3.2-4.8 cm) tumors (86.7% versus 68.8%). There were no major complications or treatment-related deaths, but minor complications (local pain, fever, mild self-limited hemothorax) were observed in 18 patients (58%). During a mean follow-up of 9 months, local tumor recurrence developed in one patient (3.3%), new intrahepatic recurrence in 3(10%), both local and new intrahepatic recurrence in another 3 (10%), but 23 patients (76.7%) remained apparently disease free.

Conclusions: In patients with cirrhosis and HCC, RFA appears to be an effective, well-tolerated, safe, and relatively simple procedure for the treatment of small and medium HCC lesions.

PP-111

Fibrosure, apri and forns scores versus liver biobsey in chronic HCV infection in Eygpt

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Intrroduction: Chronic infection with HCV a major health problem .Clinical management of chronic HCV dependent on extent liver fibrosis .Liver biopsy gold stander an invasive procedure responsible for severe complications and sample variability interpretation. Serum biomarkers for inflammation/fibrosis investigated to wave liver biopsy.

Aim: Diagnostic accuracy of panel of Non-invasive serum biomarkers for hepatic fibrosis

[Fibrosure, APRI score, Forn's score] versus liver biopsy.

Methods: 20 HCV patients subjected for: APRI, Forn's , Fibrosure scores

PCR quantitative HCV-RNA

Liver functions.

Lipid Profile

CBC.

Ultrasound guided liver biopsy.

Results:

-FORNS score; AUROC(0.917) with 95% CI(0.791-1.042) for(f_0f_1) vs. ($f_2f_3f_4$) while(0.688)with 95% CI(0.464- 0.91)for($f_0f_1f_2$) vs.(f_3f_4). Cutoff (>6.9) sensitivity for significant fibrosis ($f_2f_3f_4$) and extensive fibrosis (f_3f_4) were (100%) specificity (0%) with accuracy (40%) and (20%) respectively.

-APRI score; AUROC(0.792)with 95% CI(0.568 – 1.015)comparing(f_0f_1) vs.($f_2f_3f_4$)while was(0.875)with 95% CI (0.703 – 1.047) for ($f_0f_1f_2$) vs.(f_3f_4).Cutoff (<0.5) sensitivity (0%) and specificity (100%) with accuracy (60%) for significant fibrosis and(80%)for extensive fibrosis.

-Fibrosure (fibro-acti test); showed best AUROC (1.00) in different fibrotic stages with 95 % CI (1.00–1.00) .Cutoff (>0.59) sensitivity (50%) for significant fibrosis and (100%) for extensive fibrosis while specificity (100%) in all fibrotic stages. The PPV (100%) for significant and extensive fibrosis .NPV and accuracy (75%, 80%) respectively for significant fibroses, while NPV and accuracy (100%) for extensive fibrosis.

Significant correlation between liver biopsy and Fibro-test(P0.002)and Acti-test(P0.000).

Significant correlation between liver biopsy hepatitis activity score and APRI (P 0.047) and FORNS score (P0.000).

Conclusion:

FORNS score wasn't considered since does not discriminate between significant and extensive fibrosis. Low sensitivity of **APRI** prohibtes detection of minmal fibrosis and allow undetermined results.

Fibrosure classified all cases of chronic HCV sufficient to wave liver biopsy

PP-112

Wnt/β-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells

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Adult hepatic progenitor cells are facultative stem cells in the liver, which participates in a range of human liver diseases. In this study, we investigated whether Wnt/β-catenin signaling contributes to activation of hepatic progenitor cells in both rodent models and human hepatocellular carcinomas (HCCs). By using the AAF/PH and DDC model, we provide direct evidence that active Wnt/β-catenin signaling occurs preferentially within the oval cell population. Furthermore, Wnt/β-catenin signaling promotes expansion of hepatic progenitor cell population both when overexpressed in transplanted rat oval cells and when transiently expressed in adult mice. More importantly, we define a subpopulation of less differentiated human HCC cells that are enriched by excessive activation of Wnt/β-catenin signaling. These HCC cells possess a greater ability to form tumor *in vivo* and show a substantial resistance to standard chemotherapy. In addition, elimination of β-catenin virtually abrogated this chemoresistant cell population endowed with HCC progenitor-like features. These results highlight the importance of the Wnt/β-catenin pathway in activation and expansion of hepatic progenitor cells in normal rodent models and human HCCs. Therapies targeted to the Wnt/β-catenin signaling may provide a specific method to disrupt this resistance mechanism to improve overall tumour control with chemotherapy.

PP-113

Potential application of proteasome inhibitor bortezomib for treatment of hepatocellular carcinoma

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Despite the broad anti-tumor potential of the proteasome inhibitor bortezomib, few informations are available with regard to its efficacy as anti-hepatocellular carcinoma (HCC) agent. Here we studied the effects of bortezomib on HepG2 and JHH6, two human HCC cell lines displaying a different phenotype, hepatocyte-like for HepG2 and undifferentiated for JHH6.

Cell necrosis/apoptosis were studied by LDH, MTT, annexin V assays, cell debris counting; cell cycle phase distributions were evaluated by flow-cytometry together with the expression levels of different G1-S phase mediators.

In both cell lines, bortezomib induced a dose-dependent increase in cellular debris and in the amount of LDH together with a dose-dependent reduction in cell viability (MTT) and cell number. Whereas bortezomib-induced apoptosis was present (annexin V), more evident was the anti-proliferative effect characterized by a marked decrease of S phase and an increase of G2-M phase cells. In HepG2 this was accompanied by the reduction of the levels of cyclin D1, of

E2F1, of the hyper-phosphorylated form of pRB and cyclin A1 with an increase of $p21^{waf1/cip1}$ and $p27^{kip1}$ and LRH-1. In JHH6 most evident was the reduction of E2F1, cyclin A1 and LRH-1 with an increase of $p21^{waf1/cip1}$. Finally, compared to HepG2, an overall minor sensitivity of JHH6 to bortezomib effects was observed.
In conclusion, despite a certain phenotype-dependent effect, the cytotoxic/anti-proliferative effect exerted by bortezomib in the HCC cells considered together with the reported negligible toxicity in normal hepatocytes, confers to bortezomib the potential to become an attractive tool for HCC treatment.

PP-114
Treatment of malignant liver tumors with percutaneous microwave ablation: Experience with complications over 13 years
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Purpose: To report the incidence of complications for microwave ablation (MWA) of malignant liver tumors in a large series of patients.
Patients and Methods: Over a 13-year period, 1136 patients underwent ultrasound-guided MWA (583 with non-cooled shaft antenna and 553 with cooled-shaft antenna) for treatment of malignant liver tumors. Treatment-related major and minor complications were documented.
Results: Two deaths within 30 days after MWA were encountered. Major complications occurred in 30 patients (2.64%), which included liver abscess in 4 cases (0.35%), bile duct injury in 2 cases (0.18%), colon poforation in 2 cases (0.18%), tumor seeding in 5 cases(0.44%), pleural effusion requiring throcacentesis in 12 cases (1.06%), hemorrahage requiring arterial embolization in one cases(0.09%), and skin burn requiring resection in 3 cases (0.27%). Minor complications included fever, pain asymptomatic pleural effusions, self-limiting intraperitoneal bleeding, subcapsular hematoma and pleural effusion requiring no throcacentesis. Use of non-cooled shaft antenna and increased number of MWA sessions were associated with a higher rate of major complications ($p<.05$).
Conclusion: Microwave ablation of liver liver tumor is a well-tolerated technique with an acceptably low rate of major complications, but caution should be taken when treating tumors near the liver hilum and colon. Intraoperative thermal monitoring may help to minimize complications while maintaining therapeutic efficacy.

PP-115
Herbal compound extract "Songyou Yin" inhibits tumor growth and prolongs survival in nude mice bearing human hepatocellular carcinoma xenograft with high metastatic potential
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Objectives: To evaluate the antitumor effect of the herbal compound extract "Songyou Yin," which contains Tanshinone and another four herbs (Grant No.G20070160).
Methods: Human hepatocellular carcinoma (HCC) cell line MHCC97H with high metastatic potential was employed for in vitro study for cell proliferation, apoptosis, and invasion. In vivo study was conducted in nude mice bearing HCC xenograft with MHCC97H. The statistical SAS 8.2 software package was involved in data analysis.
Results: In vitro, "Songyou Yin" could cause dramatic attenuation of tumor proliferation by induction of cell apoptosis that was associated with caspase-3 activation, and it inhibited HCC invasion via reducing matrix metalloproteinase-2 (MMP2) activity. In vivo, "Songyou Yin" could avert cancer-related body weight loss of mice with tumors without distinct toxicity. In addition, inhibition of tumor growth was observed with stepwise increased dosage of "Songyou Yin" and accorded with the expression of proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF), and MMP2 in tumor-implanted sites. Tumor inhibition rate in the treated group reached 53.7%. Moreover, the lung metastatic extent was decreased in the "Songyou Yin"-treated group ($p < 0.01$, compared with control), and the life span of nude mice bearing orthotopic xenografts was extended in the treated group ($p < 0.01$, compared with untreated), being 75.0 ± 3.9 days versus 52.0 ± 2.3 days. Conclusion: "Songyou Yin" inhibited tumor growth that was associated with an increased TUNEL-positive apoptosis as well as a decreased microvessel density (MVD) and VEGF abundance, and inhibited HCC invasion via MMP2 down-regulation.

PP-116
Preoperative serum prealbumin level as indictor for hepatic dysfunction after liver resection in Child-A patients
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Background: Although prealbumin potentially indicates a preserved liver function, the clinical significance of pre-operative serum prealbumin is not fully understood.
Methods: Three hundred thirty seven patients received hepatectomy for various indications by a same surgical team. The patients' data was retrieved from our prospectively collected databank. Univariate and multivariate analysis were performed to study the relation between pre-operative prealbumin and post-operative hepatic dysfunction.
Results: Sixty-one (18.1%) patients had post-operative hepatic dysfunction. The average prealbumin level was 0.21 g/L. In univariate analysis, serum total bilirubin, albumin, ALT, GGT, PT, HBV infection, cirrhosis, major resection, intraoprative blood loss, perioperative transfusions were associated with postoperative hepatic dysfunction. However, the independent risk factors were prealbumin concentration, total bilirubin concentration and major resection. Patients with a prealbumin level lower than N experienced an increased incidence of postoperative hepatic dysfunction (P <0.001).
Conclusions: These results indicate that preoperative prealbumin level is a reliable predictor of hepatic dysfunction following liver resection in Child-Pugh A patients.

PP-117
Quantitative analysis of angiogenesis in hepatocellular carcinoma with contrast-enhanced ultrasonography
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Purpose: To investigate the relationship between quantitative parameters with real time gray-scale contrast-enhanced ultrasonography (CEUS) and angiogenesis in hepatocellular carcinoma (HCC).
Materials and Methods: Thirty-one patients with HCC confirmed by pathology received CEUS preoperatively.The dynamic images were recorded on cine clips and downloaded for off-line analysis. Tumor and hepatic tissue were chosen as the region of interests (ROI) to determine parameters from the time-intensity curves (TIC) fitted by gamma function: rate of 50% contrast material washout (a2), rate of signal intensity increase to initial peak (a3), increase signal intensity (△SI), area under the curve (AUC) and perfusion coefficient (P). Microvessel density (MVD) was determined by the pixel percent of intratumoral endothelial cell (CD34) at a magnification of ×100. T test was used to evaluate potential differences in these CEUS parameters and Spearman correlation test was used to investigate the relationship between CEUS parameters and histological markers.
Results: The values of a3, △SI, AUC and P of total tumor section were lower than those of hypervascular area in tumor (P<0.05). Standardized AUC and P of small lesions (≤3cm) were lower than those of large lesions (>3cm) (P<0.05). Standardized P was correlated with microscopic invasion of portal vein and/or intrahepatic metastasis (P<0.05) . The values of standardized △SI, AUC and P were positively correlated with MVD (P<0.01), and the correlation coefficient were 0.508, 0.651 and 0.676, respectively.
Conclusion: CEUS, a noninvasive imaging method, is promising in quantitative assessment of angiogenesis in HCC.

PP-118
Antitumor effect of adenovirus haboring hTERT-targeting trans-splicing ribozyme and liver specific promoter in hepatocellular carcinoma in vivo
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Advanced or multinodular hepatocellular carcinomas (HCC), distant metastasis, recurred tumors are fatal and have been required to develop new therapeutic modalities. In this study, we constructed adenoviral vectors containing cancer specific hTERT targeting *trans*-splicing ribozyme (TSR) with downstream lacZ gene or therapeutic suicidal gene HSVtk under the control of liver-specific PEPCK or ubiquitous CMV promoter. We maintained hTERT(+) Hep3B and HepG2 HCC cells, and hTERT(-) THLE3 normal liver cell. For carcinoma peritonei model, intraperitoneal injection of 2x 10^7 Hep3B cells in nude mice was done, resulting above 95% of success rate within 21 days. For specific transgenic expression, X-gal staining of in toto and frozen sections of normal tissue and tumors was done. For antitumor effect, 1x10^9 pfu of Ad-MOCK, Ad-PEPCK.Ribo.Tk and Ad-CMV.Ribo.Tk (n=10, each) on 18th day, intrapeitoneally, following injection of gancyclovir for 10 days. Eighteen days after virus injection, total weights of removed tumors were measured. For hTERT expression levels, RT-PCR and immunohistochemistry were performed. We observed tumor specific expression of B-galactosidase in Ad-PEPCK.Ribo.LacZ group and both liver and tumor in Ad-PEPCK.LacZ group. Mean tumor weights (mg) of control, Ad-PEPCK.Ribo.Tk and Ad-CMV.Ribo.Tk groups were 8.26±2.97, 3.91±1.66 and 2.36±1.39, showing significantly reduced tumor growth in treated groups (Mann-Whitney test, PEPCK; 0.0016, CMV; 0.0006). In addition, significant reduction of hTERT RNA (≈ 75%) and protein expression were observed in HCC mouse model by the specific ribozyme. These results showed that hTERT targeting TSR with PEPCK promoter represents a powerful dual targeting and dual efficacy agent for HCC targeted gene therapy.

PP-119

Clinical impacts of circulating transforming growth factor (TGF)-β1 and TGF-β1 mRNA in diagnosis of hepatocellular carcinoma

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Objective: to investigate the characteristics of TGF-β1 and TGF-β1-mRNA expression, their relationship with HBV replication, and their diagnostic values for hepatocellular carcinoma (HCC).

Methods: Total RNAs were extracted from HCC tissues and their matched non-tumor tissues, or from peripheral blood mononuclear cells (PBMCs) in HCC patients. TGF-β1mRNA were amplified by RT-PCR and confirmed by DND sequencing. Distribution of TGF-β1 expression was examined by immunohisto- chemistry. The clinical characteristics were analyzed between TGF-β1 and HBV replication. Diagnostic values of circulating TGF-β1 and TGF-β1mRNA were investigated in HCC patients.

Results: The incidence of hepatic TGF-β1 expression was 83.3% in HCC tissues, 43.3% in their surrounding tissues, 94.7% in HBV-DNA-positive group, and 63.6% in HBV-DNA-negative one, respectively. Liver TGF-β1 expression were associated with the degree of HCC differentiation and the statue of HBV replication, but neither to size nor to number of tumors. Circulating TGF-β1 level or incidence of TGF-β1 mRNA was significantly higher in HCC group than any group of patients with benign liver diseases, with higher sensitivity (89.5%) and specificity (94.0%) for HCC diagnosis when circulating TGF-β1 level (> 1.2 μg/L). No significant correlation was found between TGF-β1 expression and AFP levels or tumor sizes. Combined TGF-β1 level and serum AFP could raise the detection rate up to 97.4%.

Conclusion: The abnormal expression of hepatic TGF-β1 was associated with degree of HCC differentiation and HBV replication. Both of circulating TGF-β1 and TGF-β1-mRNA could be used as sensitive biomarkers for diagnosis and prognosis of HBV-induced HCC.

PP-120

Mechanism of alcohol-induced RNA pol III gene transcription

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Alcohol abuse is a major cause of liver fibrosis. Eventually, alcohol-induced liver fibrosis promotes formation of liver cirrhosis, which increases the risk of liver cancer. Cancer cells have a consistent cytological feature of nucleolar hypertrophy, enlarged nucleoli, where rRNAs are synthesized by RNA pol I and pol III. This implies that transformation in situ is closely linked to the deregulation of RNA pol I- and III-dependent transcription, because the size of the nucleolus reflects the levels of rRNA synthesis. RNA pol III genes encode a variety of untranslated RNAs, including tRNAs and 5S rRNAs. Deregulation of RNA pol III-dependent transcription, enhancing cellular tRNAs and rRNAs production, leads to an increase in translational capacity to promote cell transformation. Although alcohol has been widely studied, nothing is yet known as to whether the transcription of RNA pol III-dependent genes might be affected by alcohol. To explore the mechanism of alcohol-associated liver cancer, we have investigated whether alcohol induces RNA pol III transcription. Results indicate that ethanol induces transcription of RNA pol III genes in engineering HepG2-ADH cells and liver of mouse fed with ethanol. Further analysis reveals that ethanol increases TBP promoter activity and enhances cellular level of TBP, which is a limiting factor of RNA pol III transcription. Ethanol treatment stimulates activation of JNK1. Our previous study has indicated that JNK1 positively regulate TBP expression. Together, these results demonstrate that alcohol induces TBP expression, resulting in increase of RNA pol III gene transcription through JNK1 signaling pathway.

PP-121

The role of CEUS in evaluating therapeutic efficacy of local treatment of hepatic malignancies

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Objective: To delineate CEUS appearance of hepatic tumors after local treatment and to summrize the CEUS pattern of viable tumor at the site of local treatment.

Method: Between January 2005 and July 2007, 116 patients with 116 tumors (95 hepatic primary carcinoma and 21 metastases) treated with local treatment underwent CEUS and CECT imagings during follow-up period at the same time.

Result: The gold diagnostic standard was erected synthetically by 2 contrast enhanced imagings，combined with the level of tumor marker, biopsy and more than 6 months' follow-up. There were 5 patterns of enhanced appearance of viable tumor on CEUS, included nodular, washing out, mass-like, ring-like, irregular appearance, respectively. CEUS had good correlation with CECT on tumor size (r=0.75 p<0.0001) and appearance (Kappa=0.82(Z=12.75 p<0.0001)). Areas under the ROC of CEUS and CECT were 0.956 and 0.934 respectively and had no significant difference (Z=0.095，P=0.92). The sensitivity , speciality and accuracy was 98%、93.9%、95.7% for CEUS, 88.4%、98.4%、94.3% for CECT without significant difference.

Conclusions: CEUS can accurately differentiated viable tumor from complete necrotic tumors after local treatment of hepatic malignancies. CEUS play an important role in evaluating therapeutic response for liver malignancies because of high correlation with CECT.

PP-122

Laparoscopic versus open liver resection for hepatocellular carcinoma: a comparative study

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Background: Laparoscopic liver resection for hepatocarcinoma could reduce morbidity, especially in cirrhotic patients. However, most previous studies report a majority of atypical resections of the liver. Our aim was to compare the postoperative results and mid-term outcomes between laparoscopic (LLR) and open liver resection (OLR) for hepatocarcinoma.

Methods: Between 1999 and 2007, we performed 40 laparoscopic resections for hepatocarcinoma. The selection criteria were: tumor < 7cm, well-compensated cirrhosis and ASA score < 3. An anatomical resection was preferred when possible. Laparoscopic patients were compared retrospectively with 88 patients who underwent open surgery for hepatocarcinoma from our liver resection database. The two groups were matched for age, gender, tumor size, type of liver resection and severity of liver disease. Chi-square, Fisher exact test and Mann-Whitney test where used as appropriate.

Results: The mean operative duration was similar in both groups (LLR, 237 min; OLR, 260 min; p=0.13). Blood loss was significantly lower in LLR (393 vs. 778 ml; p<0.0001). However, the difference in transfusion rates was not statistically significant (LLR, 12%; OLR, 21%; p=0.32). There was a significant decrease of postoperative ascites in laparoscopy (5% vs. 19%; p=0.03). Hospital stay was significantly

shorter in the LLR group (6.7 vs. 11.9 days; p<0.0001). The 1- and 3-year survival rates were similar for LLR and OLR.
Conclusions: This study shows that laparoscopic liver resection for hepatocarcinoma have better postoperative results and similar oncological outcomes than open surgery.

PP-123
The influence of vitamin K2 on caspase-3 mRNA expression of MHCC97-H and on pulmonary metastasis and life span of nude mice bearing human hepatocellular carcinoma
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Objective: To study the mechanisms of apoptosis in human hepatocellular carcinoma cell strain with highly metastatic potention (MHCC97-H) induced by vitamin K2(VK2) and to observe the influence of VK2 on pulmonary metastasis and life span of nude mice bearing human hepatocellular carcinoma.
Methods: The mRNA expression of caspase-3、bcl-2、Bax was detected by RT-PCR respectively. Nude mice bearing orthotopic xenograft of human HCC were randomly divided into two groups: VK2 group($30mg\bullet kg^{-1}\bullet d^{-1}$) and control group(only vector) with administration by gavage. Their pulmonary metastases and life span were recorded.
Results: Expression of caspase-3 mRNA increased in dose- and time-dependent manner when the dose increased from 20μM/L to 100μM/L and the time increased from 24h to 72h. After treated with 100μM VK2 72 hours, there was a significant difference of expression of caspase-3 mRNA between VK2 group (0.603±0.098) and control group(0.270±0.132),P<0.05.Comparing with control group,the number of pulmonary metastases was decreased(4.6±1.95 versus 12±6.6) and life span was prolonged(83.6±5.18d versus 58.2±9.47d) in VK2 group of nude mice bearing orthotopic xenograft of human HCC, P<0.05.
Conclusions: The apoptosis of MHCC97-H cells could be induced by VK2 via caspase-3-transducted signal. VK2 also produced marked inhibitory effects on pulmonary metastasis and beneficial influence on life span of nude mice bearing orthotopic xenograft of human hepatocellular carcinoma.

PP-124
Identification of the cell clonal origin of multinodular hepatocellular carcinoma by analyzing the mitochondrial DNA D-Loop region variations
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Objective: To explore the feasibility of identifying clonal origin of hepatocellular carcinoma (HCC) by analyzing the mitochondrial DNA(mtDNA) D-Loop region variations and the clinicopathologic characteristics.
Methods: Study group (multinodular HCCs) were 42 patients. Controls were 20 HCCs that consisted of 16 single nodular HCC cases which each had two pieces of inconsecutive tumor tissues and 4 HCC cases with portal vein tumor embolus.Polymerase chain reaction (PCR) and direct sequencing were applied to study the mtDNA D-Loop region.
Results: In study group,20(47.62%) cases were categorized as multicentric occurrence(MO) based on their variant D-Loop sequences in each nodule from the same patient.While 22 cases in study group and 20 cases in the control group were characterized as intrahepatic metastasis(IM) based on the identical D-Loop sequences found in each tumor tissue from the same patient.Positive HBeAg（P=0.008），cumulative diameter of all nodules≤7cm（P=0.029），nodules located in different lobes（P=0.041），cirrhosis（P=0.011），without portal vein or microscope tumor embolus（P=0.023） and/or well/moderate differentiation of main nodular histopathology（P=0.026） were attributed to a high rate of MO.Both Tumor-free survival and overall survival of the MO subjects were significantly longer than that of the IM subjects (P=0.022,P=0.006 respectively).Multivariate analysis revealed that the IM/MO characteristic was an independent factor for either tumor-free survival(P=0.012) or overall survival(P=0.011).
Conclusions: 1.Sequencing the mtDNA D-Loop and analyzing the clinicopathologic characteristics provide a tool for the determination of clonality of the multinodular HCCs. 2. MO HCC patients might have a favorable outcome compared with IM patients.

PP-125
Safety profiles of sorafenib in the treatment of metastatic hepatocellular carcinoma
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Objective: To analyze the safety profile of sorafenib in the treatment of metastatic hepatocellular carcinoma (HCC) patients.
Patients and methods: From Aug 2007 to Jan 2008, 11 metastatic HCC patients (100% male, median age of 52 years old, median PS of 1) were treated with sorafenib in the department of medical oncology, Zhong Shan Hospital, Fu Dan University. , Sorafenib were given as 400 mg Bid po. Dosage reduced to 200 mg Bid when patients manifestated any untolerated symptoms. Adverse events were evaluated according to NCI-CTC 2.0 version.
Results: Among 11 patients, 6 (55%) had good liver function with Child A, 4 with Child B, 1 with Child C. Median treatment time was 30 day. 9 patients (91%) used sorafenib alone, 2 patients (9%) were treated combined with chemotherapy. Fatigue occurred in all patients, only 1 patient (9%）ranked grade 3 . 4 patients (36 %) had grade 1 or 2 dermatologic toxicity, such as alopecia, desquamation and hand-foot syndrom. Gastrointestinal toxicity, anorexia (82 %) and stomatitis (18 %) were limited to grade 1 or 2. Liver function impairment happened in 2 patients (18 %). 3 patients (33%) had grade 1 haemorrhage with epistaxis. 1 patient had unexplained back pain. 2 patients had hoarsness.
Conclusion: Sorafenib as a new target agent, was well tolerated with less grade 3 adverse events occurred in late stage of HCC patients. However, highly selected and careful observation are recommended while considering sorafenib in treating HCC patients.

PP-126
The mechanism of the effect of interferon alpha on VEGF transcription and angiogenesis
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Objective: To evaluate the role of transcription factors Stat3 in transcription control of Vescular endothelial growth factor (VEGF) in human hepatocellular carcinoma.
Methods: Contructing Reporter Plasmids and Mutagenesis containing VEGF promoter, transfecting to human hepatocellular carcinoma cell line MHCC97H, and analyzing the critical regions of VEGF transcriptional control, and the role of Stat3 on transcription control of VEGF. electrophoretic mobility shift assays (EMSA) were used to determine the binding activity of Stat3, and knockdown the expression of Stat3 by RNAi to analyze the role of Stat3 expression in the transcription control of VEGF**.**
Results: A series of 5' deletion mutants of VEGF promoter reporter gene based on the 2274-bp VEGF promoter were transfected into MHCC97H cells, and examined VEGF promoter activity. The result showed that the -109/-61 region contains essential regulatory elements. Point mutagenesis of transcription factor binding sites showed that eliminated the inhibitory effect of IFN- on VEGF promoter activity, whereas such effect was not found in mutation of either AP-2 or Egr-1 sites. It is suggested that Stat3 binding sites in this region are responsible for VEGF promoter activity. using RNA interference technique to reduce expression level, and found that VEGF promoter activity was almost eliminated. **Conclusion:** Transcription factor Stat3 binding VEGF promoter plays a crucial role in the transcription control of VEGF, and associate with metastatic potential of hepatocellular carcinoma.

PP-127
Diagnostic values of combined hepatoma-specific α-fetoprotein (AFP) fraction and telomerase in patients with hepatocellular carcinma
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Objective: To explore the diagnostic values of hepatoma- specific alpha- fetoprotein fraction (AFP-L3) and telomerase patients with hepatocellular carcinoma (HCC).
Methods: Total RNAs were extracted from circulating blood, synthesized to cDNA through random primers and reverse transcriptase, the telomerase activities of peripheral blood mononuclear cells （PBMCs）were analyzed by TRAP-ELISA. AFP-L3 was separated by lectin-affinity chromatography and both of AFP and AFP-L3 were detected by radioimmunoassay (RIA).

Results: Of patients with HCC, cirrhosis, chronic hepatitis, and normal control group, the incidences of AFP-L3% were 90.9%, 20%,7.5% and 0; and telomerase positive rate were 74.5%, 16.4%, 10.0% and 0%, respectively. The incidence of AFP-L3% or telomerase in HCC group was significantly higher (P<0.01) than that in cirrhosis, chronic hepatitis and normal control group. No significant relationship was found between AFP-L3% or telomerase and Total AFP, except of HCC metastasis and relapse.
Conclusion: The analysis of AFP-L3% or telomerase are useful markers to diagnose and differentiate HCC or monitor metastasis and relapse of HCC.

PP-128
In vitro and in vivo targeted imaging of hepatocellular carcinoma by quantum dot probes
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Semiconductor quantum dots (QDs) have several photo-physical advantages better than organic dyes which make them good markers in a wide application in biomedical field. We used CdSe/ZnS core/shell quantum dots (QDs) with maximum emission wavelength of 590 nm (QD590) linked to alpha-fetoprotein (AFP) monoclonal antibody (Ab) to form QD-AFP-Ab probes for the imaging of human hepatocellular carcinoma (HCC) cell line HCCLM6 *in vitro*. Then the QD-AFP-Ab probes were injected into the tail vein of tumor bearing nude mice for targeting and imaging of HCC growth and metastasis *in vivo*. The three-dimension (3D) analysis of QD signals distribution characteristics *in vivo* showed that the probes per field were lower in the centre than in the periphery of the tumor. Successful *in vitro* and *in vivo* imaging of HCC and its lung metastases were obtained. In addition, potential toxicity profile, the non-specific uptake and distribution in different normal host organs were also studied. All these results suggest this QD-based probe could be useful for molecular imaging of AFP-producing HCC.

PP-129
Percutaneous radiofrequency ablation for the treatment of hepatocellular carcinoma in the caudate lobe
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Purpose: To evaluate the efficacy and safety of Percutaneous Radiofrequency Ablation (PRFA) for hepatocellular carcinoma (HCC) in the caudate lobe.
Materials and Methods: 17 patients with 20 HCCs were treated with either PRFA alone (n=14), or PRFA with percutaneous ethanol injection (n=3) under ultrasound (US) guidance. The right or the anterior approach was used in 12 and 5 patients, respectively.
Results: 14 of 17 patients had their tumors completely ablated after one to two sessions of treatment. Two developed local recurrence, Intrahepatic metastasis developed in 9 of 17 patitents. No distant metastasis was seen. 4 of the 17 patients died, tumor progression (n=3) and hepatic failure (n=1). There was no major complication in the study.
Conclusion: PRFA is efficacious and safe for patients with HCC in the caudate lobe

PP-130
The role of contrast enhanced ultrasound in radiofrequency ablation of liver metastasis
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Purpose: To evaluate the clinical value of contrast enhanced ultrasound (CEUS) for radiofrequency ablation (RFA) of liver metastasis.
Materials and Methods: 141 patients with liver metastasis were referred to RFA in our department. Of them, 102 received CEUS before RFA treatment. 16 patients were excluded from RFA after CEUS and the remaining 86 patients were treated based on the result of RFA (Group A). During the same period, another 39 patients without contrast before RFA were served as the control group (Group B).
Results: CEUS demonstrated 49.7% (75/151tumors) were larger in size and detected additional 1-3 tumors in 36 patients (41.9%) compared with conventional US. The RFA protocol for each case was designed according to CEUS finding. The tumor necrosis rate in Group A was 94.7% (198/209 tumors), which was significantly higher than 87.6% (99/113 tumors) in Group B (P=0.013). Local recurrence were found in 15 tumors (7.1%) in Group A, which was significantly lower than 16 tumors (14.1%) in Group B (P=0.041).
Conclusion: The use of CEUS can increase tumor necrosis rate and decrease post-RFA tumor local recurrence, and then improve efficacy of RFA therapy.

PP-131
IGF-II inhibits adriamycin-induced apotosis of HepG2 by up-regulating servivin expression
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Objective: To investigate the effects of IGF-Ⅱ on the adriamycin -induced apoptosis of the hepatoma carcinoma cell line(HepG2) and the expression of Survivin.
Methods: Grouping:①group A:control group;②group B:ADM group;③group C:2ng/ml IGF-Ⅱ＋200ng/ml ADM group;④group D:20ng/ml IGF-Ⅱ＋200ng/ml ADM group;⑤group E:200ng/ml IGF-Ⅱ＋200ng/ml ADM group.After HepG2 were treated with each group for 48h,MTT colorimetry was performed to determine the cell viability,Flow cytometry was used to detect the cell apoptosis rate,and Weastern-Blot was performed to evaluate the expression of survivin.
Results: The cell viability of group A、B、C、D、E is:0.568±0.025、0.201±0.020、0.232±0.027、0.268±0.013、0.304±0.019;the cell apoptosis rate of each group is :6.9%±1.3%、35.4%±2.1%、31.2%±2.2%、26.4%±1.7%、21.7%±1.9%;survivin/β-actin of each group is: 0.527±0.039、0.147±0.081、0.311±0.069、0.421±0.033、0.469±0.031.The cell viability in IGF-Ⅱ＋ADM group is notably better than ADM group(P <0.01), the cell apoptosis rate in IGF-Ⅱ＋ADM group was significantly lower than ADM group(P <0.01), the expression level of Survivin in IGF-Ⅱ＋ADM group was significantlyhigher than ADM group(P <0.01), and the above-mentioned effects were improved with the gradual ascensus of the IGF-Ⅱ concentration(P <0.05).
Conclusions: IGF-Ⅱ can restrain the ADM-induced apoptosis in HepG2 cells,decrease the sensitivity of hepatoma carcinoma cell to chemotherapeutic agents, IGF-Ⅱ may play the anti-apoptosis role by up-regulating Survivin expression in HepG2 cells。

PP-132
Identification of capn4 associated with metastasis and recurrence of hepotacellular carcinoma after liver transplantation by quantitative proteomics
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Objective: To screen the key molecule associated with metastasis and recurrence of hepatocellular carcinoma after liver transplantation.
Methods: The primary liver cancer samples of the patients with hepatocellular carcinoma after liver transplantation who have the same disease background were divided into the recurrent group and non-recurrent group (six cases each group), according to our follow-up data. To acquire the homogeneous hepatocellular carcinoma cells from HCC tissue and extract the total protein, which was labeled with the light and heavy cICAT reagent respectively, and identified with two dimensional liquid chromagraphy coupled with tandem mass spectrometry. Immunohistochemistry and western blotting were performed to examine the differential expression of the candidate proteins.
Results: Total 149 proteins including 29 up-regulated 2-folds and 23 down-regulated 2-folds proteins were identified, of these, Capn4 was found to be uniquely over-expressed in the recurrent group when compared to the non-recurrent group.
Conclusion: The metastasis and recurrence of hepatocellular carcinoma after liver transplantation may be correlated with the speicifical variation of the protein expression profile. Capn4 may serve as a potential molecular target associated with the metaststic HCCs after liver transplantation.

PP-133
Clinical value of active breathing coordinator (ABC) during three dimension conformal radiotherapy for patients with introhepatic

tumor

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Background：The purpose of our study was to measure the liver excursion using predominance of ABC in hepatocellular carcinoma patients treated with 3D-CRT .

Methods：From May 2005 to Jan 2006, 16 patients with unresectable liver cancer (9 hepatocellular carcinoma、4 metastasis mass and 3 cholangiocarcinoma) underwent 3D-CRT with ABC. Image fusion was used to measure liver excursion in three dimension due to free breathing. Interfraction and intrafraction reproducibility of liver centroid relative to the skeleton were also determined from three-dimensional alignment of repeated CT scans obtained in the treatment position. The two treatment plans based on PTV with ABC and free breathing was compared.

Results: The ABC procedure was well tolerated in the 16 patients. The average intrafraction and interfraction reproducibility of diaphragm to vertebral bodies was 1.5 mm (range, 0.6 –3.9mm) and 3.4 mm (range, 1.5–7.9 mm) . The liver centroid transformation from week-to -week CT scans was 2.38 ±1.7 mm, 2.65 ±2.5 mm, 3.69 ±3.3mm in the ML, AP,and CC directions.Significant difference was found between ABC and free breathing(FB) plan in the V30, mean hepatic dose, the maximun dose of normal liver with 10% Normal Tissue Complication Probability(NTCP).

Conclusion: ABC provides a simple mean to minimize the target motion due to breathing which minimize the volume of normal organs irradiated and facilitates dose escalation to intrahepatic tumors. However there remains some inter– breath hold variability in liver position, suggesting a need for daily image guidance to further decrease PTV margin.

PP-134

Serum metabonomic profile on hepatocellular carcinoma patients through chemical derivitization

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The purpose was to investigate the serum metabolic difference between hepatocellular carcinoma (HCC, n=20) male patients and control normal male subjects (n =20). Serum metabonome was detected through chemical derivatization followed by gas chromatography/mass spectrometry (GC/MS). Data was analyzed by stepwise discriminant analysis (SDA) and support vector machine (SVM). Through SDA, we got metabolites including butanoic acid, ethanimidic acid, glycerol, L-isoleucine, L-valine, aminomalonic acid, D-erythrose, hexadecanoic acid, octadecanoic acid, 9,12-octadecadienoic acid and so on which in combination with each other gave the strongest segregation between the two groups. Error count estimate for each group was 0%. The total classifying accuracy tested by SVM 20-fold cross validation was 75%. By applying these variables, our method provided a diagnostic model that could well discriminate between HCC and normal subjects.

PP-135

Growth inhibition in cultured human hepatocellular carcinoma cells HUH-7 and suppression of HUH-7-derived tumor development in mice by novel lipid compounds

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Successful chemotherapy of hepatocellular carcinoma (HCC) remains highly problematic due to a low specificity and/or high toxicity of approved drugs (e.g. Sorafenib). We have designed, synthesized and examined several novel compounds containing various fatty acids for their ability to inhibit HUH-7 cell proliferation/survival in culture and suppress tumor development when injected in SCID mice. Three compounds containing C16:0, C18:1 or C8:0 residues, termed STA-11, STA-21 and STA-31 respectively, exhibited a potent anti-mitogenic/anti-survival activity against cultured HUH-7 cells with IC50 of 6.85 μM, 5.3 μM and 11.5 μM, respectively. In contrast, for primary rat and human hepatocytes in culture, the IC50 for STA-1 and STA-2 were found to exceed 100 μM. The LD50 for STA-11 in wild type C57/BL mice was estimated to be 149 mg/kg after i.p. injection of the compound. All wild type mice tolerated well the multiple injections of the compound at 5 mg/kg. All SCID mice injected subcutaneously with HUH-7 cells developed massive tumors at 7 week, while co-injection of HUH-7 and STA-11 at 1.0 mg/kg did not lead to the development of visible tumors during 7 week observation.

Our data indicate that novel lipid inhibitors of HUH-7-derived malignancies developed by our group show a great promise and warrant further preclinical studies of these compounds for treatment of HCC.

The experimental details and potential mechanism (s) of inhibition of HCC growth by this class of novel compounds will be discussed.

PP-136

Effect of H22 cells treated with microwave ablation on the antigen presenting function of macrophages in vitro

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The aim of this study was to investigate whether the H22 tumor cells treated with MWA can activate the antigen presenting function of macrophages. The macrophages were divided into three groups，and co-incubated together with tumor cells suspension 0.5ml treated with MWA, untreated cells, and saline. The experiments were ended after 9h, 18h, and 27h. The expressive intensities of experimental groups of macrophages in MD-1、MHC H-$2K^b$/H-$2D^d$ and I-A^d、CD86 molecules staining were all significant increased than placebo group, and among them, the intensities of MHC H-$2K^b$/H-$2D^d$ and I-A^d molecules were highest in 9h, and MD-1 and CD86 molecules in 18h. In conclusion, the tumor cells suspension treated with MWA can activate the antigen presenting function of macrophages in mice, and the expression of MHC molecules and CD86 molecule on the surface of it are upregulated significantly.

PP-137

Contrast-enhanced ultrasonography and spiral computed tomography in the detection and characterization of portal vein thrombosis complicating hepatocellular carcinoma

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Background and aim: To compare contrast-enhanced (CE) ultrasonography (US) versus tri-phasic spiral computed tomography (CT) for detection and characterization of portal vein thrombosis complicating hepatocellular carcinoma (HCC).

Methods: We studied 50 patients with HCC accompanying cirrhosis and evidence at US and color Doppler US of thrombosis involving the main trunk of the portal vein (n=13) or its segmental branches (n=37). The nature of thrombi was defined by US-guided biopsy. CEUS and CT were performed within a week of thrombus biopsies. Imaging diagnoses of thrombosis and of its nature, made by experienced readers under blinded conditions, were evaluated in light of pathological findings.

Results: Forty-four of the portal vein thrombi were pathologically diagnosed as malignant and the remaining six were benign. CEUS detected 50/50 (100%) thrombi and characterized 49/50 (98%), while CT detected 34/50 (68%) thrombi and characterized 23/50 (46%). CEUS was superior to CT in terms of thrombus detection rates ($p< 0.0001$) and thrombus characterization rates ($p< 0.0001$).

Conclusions: CEUS appears to be significantly superior to CT for both the detection and characterization of portal vein thrombosis complicating HCC accompanying cirrhosis, and it should be considered in the staging of these tumors.

PP-138

Therapeutic effect of transcatheter arterial chemoembolization with percutaneous ethanol injection for treatment of primary stage liver cancer

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Objective: To explore the clinical effect of transcatheter arterial chemoembolization (TACE) combined with percutaneous ethanol injection (PEI) for the treatment of primary stage liver cancer after one week.

Methods: Five cases were treated with TACE plus PEI and 5 with simple TACE. Postoperative tumor size, decrease of alpha fetoprotein (AFP) and survival rate were compared.

Results: Compared with TACE group, PEI group had smaller tumor, higher recovery rate, less damage to liver function and longer survival time with less postoperative adverse effect.

Conclusion: Combined therapy of TACE plus PEI can obtain better result over simple TACE for the cases of primary stage liver cancer.

PP-139

Biological functions of ARNT on tumor growth and metastasis of hepatocellular carcinoma

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Aryl hydrocarbon receptor nuclear translocator (ARNT), an important transcription factor of bHLH/Per-ARNT-Sim family, has been reported to be involved in multiple signal pathways. ARNT may regulate chemical metabolism, cell proliferation and tumor angiogenesis during embryo develepment and tumor evolvement. Here we investigate biological functions of ARNT on tumor growth and metastasis of human hepatocellular carcinoma (HCC) using HCC cell lines, xenograft models and patient's biopsy tissues. Two specific small interfering RNA against arnt or one scramble RNA together with GFP were reconstituted into pseudo-lentiviruses and stably infected into parental HCCLM6 cell line. ARNT mRNA levels were significantly down-regulated from 100%±0% in scramble RNA treated cells (HCCLM6-C) to 14.02%±4.61% in one specific arnt interfering RNA treated cells (HCCLM6-V1) and to 40.87%±1.00% in another specific arnt interfering RNA treated cells (HCCLM6-V2), as confirmed by RT-PCR analyses. Cell population doubling time of HCCLM6-C, HCCLM6-V1 and HCCLM6-V2 were 48.7h, 30.6h and 39.1h respectively. Tumor growth of HCCLM6-V1 and HCCLM6-V2 in xenograft models in 10 nude mice were significantly accelerated compared with that of HCCLM6-C during six weeks after surgical orthotopic implantation. More metastatic foci in the lung and in the peritoneum were found in HCCLM6-V1 and in HCCLM6-V2 xenograft models compared with that in the HCCLM6-C xenograft model. Furthermore, ARNT expression in 131 HCC biopsy tissues using tissue microarray and immunohistochemistry staining revealed that HCC patients with positive ARNT expression had statistically long survival time in comparison with the ones with negative ARNT expression, and a negative correlation between ARNT expression and tumor size were found. In conclusion, high level expression of ARNT in HCC may inhibit the growth and metastasis of tumor. Thus, ARNT is thought to be a tumor suppressor gene on HCC growth and metastasis.

PP-140

Surgical outcome of different types of tumor thrombi in portal vein in hepatocellular carcinoma patients

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Background: The role of surgical resection for HCC with PVTT in different branches of portal vein is controversial. The purpose of this study was to assess the therapeutic effects of surgical resection for PVTT of HCC in different types.

Methods：From January 2000 to December 2003, 406 patients of HCC with PVTT underwent hepatectomy and thrombectomy in our hospital were retrospectively analyzed. All the patients were divided into I~IV types by the tumor thrombi type system.

Results: The median survival times were 22, 15, 10 and 10 months for type Ⅰ, type Ⅱ, type Ⅲ and type Ⅳ, respectively. The 1-, 2- and 3-year survival rates were 44.7%, 26.3% and 19.7% for type Ⅰ, 29.9%, 20.6% and 12.4% for type Ⅱ, 25.0%, 12.5% and 3.6% for type Ⅲ, 27.3%, 0% and 0% for type Ⅳ, respectively. Significant difference appeared in the survival time among the type Ⅰ, Ⅱ and Ⅲ($P < 0.05$). There was no difference between the type Ⅲ and Ⅳ($P>0.05$).

Conclusion: As compared with the control groups, we conclude that surgery remains the most effective treatment for HCC with PVTT on type Ⅰ and type Ⅱ. No significant difference appeared in the survival on type Ⅲ and Ⅳ. The new tumor thrombi system might have guiding function to determine treatment and prognosis of HCC with PVTT.

PP-141

Suppressing hypoxia-inducible NDRG1 expression sensitizes hepatocellular carcinoma cells to doxorubicin cytotoxicity.

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Background & Aims: Hypoxia may activate survival signals in cancer cells including hepatocellular carcinomas (HCCs). Hypoxic cells are less sensitive than normoxic cells to doxorubicin cytotoxicity, which is a potent activator of tumor suppressor gene p53. N-myc downstream-regulated gene-1 (NDRG1) is a hypoxia-inducible protein and has been implicated in carcinogenesis. Since this protein is also a downstream target of p53, we attempted to examine if suppressing NDRG1 expression may sensitize hypoxic HCC cells to doxorubicin cytotoxicity.

Methods: Huh-7 cells, a human HCC cell line, were used in this study. NDRG1-specific siRNA was used to suppress NDRG1 expression. Apoptotic cell death was measured by DAPI staining, and apoptotic and kinase signaling pathways were explored by immunoblot analysis.

Results: HCC cells expressed NDRG1 protein, and its expression was increased in cells cultured under hypoxic condition as compared to normoxic cells. This hypoxia-inducibility was mediated via HIF-1-dependent pathway. Doxorubicin treatment induced HCC cell cytotoxicity by activating mitochondrial apoptotic signals, such as caspase 9 activation. Suppressing NDRG1 expression enhanced doxorubicin-induced HCC cell apoptosis, and this enhancement was due to more augmented activation of JNK and caspase 9.

Conclusions: These results demonstrate that hypoxia induces NDRG1 expression in HCC cells, and that this induction leads to doxorubicin resistance in hypoxic cells. Thus, the selective interruption of NDRG1 signaling may be therapeutically useful in HCCs, especially in advanced infiltrative type of tumors which are exposed to hypoxic environment.

PP-142

Detection of helicobacter species 16S rRNA gene in liver tissues from patients with various etiologies of liver diseases

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Background: We investigated the relation between Helicobacter species and liver diseases.

Methods: Thirty-four surgically excised tissues from patients with HCC and the biopsy from liver diseases with hepatitis B virus (n=26), C virus (n=13), B and C dual infection (n=5) and fatty liver disease (n=22), were examined by polymerase chain reaction (PCR) with Helicobacter specific 16S rRNA primers. Besides, other genes (vacA, cagA) specific for Helicobacter pylori (H.pylori) were also detected by PCR. PCR products of positive samples were further identified by Southern hybridization and DNA sequencing. The presence of Helicobacter species was detected by in situ hybridization to confirm the type of Helicobacter.

Results: Helicobacter species 16S rRNA was found in 64.71% (22/34) of liver samples from patients with HCC , while the other groups were negative ($P < 0.01$).The PCR-amplified products were identified by Southern hybridization and sequencing. The homology to 16S rRNA of H pylori was 97.80%. The samples were verified by in situ hybridization for Helicobacter species 16S rRNA-mRNA and proved to be H pylori positive. Only 3 HCC samples of the cagA genes were detected. None of the samples reacted with primers for vacA in HCC groups. Conclusions: We have found that H. pylori infection is much less prevalent in benign liver diseases. The presence of 16S rRNA of Helicobacter species in liver tissues was associated with human hepatic carcinogenesis.

PP-143

13 year review of the use of adjuvant therapy (AT) both pre and post liver transplant (LTx) shows improved recurrence-free survival for liver cancer when compared to no treatment (Rx) in those that met or were beyond Milan Criteria.

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[1] *Integris Nazih Zuhdi Transplantation Institute*

Liver Cancer Adjuvant Therapy(AT) before and after LTx is controversial.

Methods: Of 80 patients with LT's over 13 years for HCC, 55 had trans-arterial chemoembolization (TACE, percutaneous ethanol injection (PEI) (tumor ≤2cm) or radiofrequency ablation RFA (tumor >2cm) while awaiting LT. 21 of them received doxorubicin and cisplatin IV every 4 weeks for 4 cycles post LTx. . 34 of 55 received only pre-LT Rx(Group B); 21 received both pre- and post-Rx(Group BA) 25 did not received pre-Rx , incidental HCC/Refused of which 4 had only Post LTx CT and 21 had no Rx.(Group NN). Kaplan-Meier recurrence free survival was measured.

Results: Average time to recurrence was 12.7m, 15.8m, 50.5m and 35.7m. Patients with post-transplant CT (Group A and BA) had longer time to recurrence. In Group NN, 13 met MC; 8 were beyond MC. In Group B, 25 met MC; 9 were beyond MC. In Group BA, 12 met MC; 9 were beyond MC. Those patients with HCC meeting MC , 5-year survival in Group NN, B, BA was 74%, 84%, 100% MC patients, 1-year survival in Group NN, B, BA was 45%, 54.7%, 100.0%; 3-year survival was 22.5%, 54.7%, 66.7%. Overall Group BA had a significant better survival than Group NN ($p<0.05$).
Conclusion: Using adjuvant therapy both Pre-LT and Post-LT CT for HCC delayed the onset of recurrence and significantly improves survival when compared with no therapy in cancers that met or were beyond Milan criteria.

PP-144
Survivin promotes the malignant phenotypes of human hepatocellular carcinoma cell line SMMC-7721
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Objective: To study the effects of survivin on the phenotypes of human hepatocelular carcinoma cel1 line SMMC-7721.
Methods: Human hepatocellular carcinoma SMMC-7721 cells were transfected with plasmid pEGFPC1/survivin and cells transfected with a mock plasmid served as controls. Survivin expression was verified by Realtime-PCR and Western blot. A series of functional assays in vitro were used to monitor the changes of SMMC-7721 malignant phenotypes · SMMC-7721 stably expressing high level of survivin was established by G418 screening. The expression differences of metastasis-related genes between two groups were compared by gene chips.
Results: Survivin expression of SMMC-7721 cells was elevated after transfection · Functional assays in vitro indicated that cells after transfection showed higher adhesive, migrant and invasive capabilities than those of the controls. 16 metastasis-related genes of SMMC-7721 highly expressing survivin were up-regulated, while 2 genes were down-regulated.
Conclusion: Survivin might promote malignant phenotypes of SMMC-7721 cells.

PP-145
Expression and significance of polar regulative associated protein aPKC-ι in hepatocellular carcinoma
Li Qiang[1], Wang Jian-ming[1], Lu Jin-xi[1], Du Guang-sheng[1], Zou Sheng-quan[1]
[1] *Huazhong University of Science and Technology Tongji Medical CollegeTonghi Hospital*
Objective: This study was to investigate the expression of aPKC-ι in hepatocellular carcinoma (HCC), and analyze the correlation of the expression of aPKC-ι in HCC and clinicopathological characteristics of HCC, and investigate molecular mechanisms of the invasion and metastasis of HCC.
Methods: The expression of aPKC-ι in normal liver tissues and HCC was detected by RT-PCR and immunohistochemistry.
Results: The positive rate of aPKC-ι gene was significantly higher in HCC than in normal liver tissues. The positive expression intensity of aPKC-ι in HCC was remarkably stronger than that in normal liver tissues (65.9% vs. 18.2%), while the expression intensity of E-cadherin was weaker in HCC than that in normal liver tissues (53.7% vs. 9.1%). Correlation analysis revealed that the expression of aPKC-ι was positively related to the progress of the tumor pathological differentiation and invasion. Moreover, there was a negative relationship between the expression of aPKC-ι and that of E-cadherin(r =-0.236, P =0.015).
Conclusion: The expression of aPKC-ι may reflect the differentiation and invasive potential of HCC. As a polar regulation-associated protein, aPKC-ι may play an important role in the invasion and metastasis of HCC.

PP-146
Biological characteristics of fluorescent protein-expressing human HCC xenograft model in nude mice
Bi-wei Yang[1], Ying Liang[1], Hui-chuan Sun[1], Lu Wang[1], Ju-bo Zhang[1], Zhao-you Tang[1], Kang-da Liu[1], Jie Chen[1], Qiong Xue[1], Jun Chen[1], Dong-mei Gao[1], Wei-zhong Wu[1], Jing-lin Xia[1]
[1] *Liver Cancer Institute and Zhongshan Hospital, Fudan University, Shanghai 200032, China*
Purpose: To study biological characteristics of stable RFP- or GFP-expressing HCCLM3 cell lines and those of their relevant xenograft models in nude mice.
Methods: HCCLM3, a human HCC cell line with high metastatic potential was infected with RFP or GFP full-length cDNA via lentivirus. Stable RFP- or GFP-expressing HCCLM3 cells, namely HCCLM3-R and HCCLM3-G, were subcutaneously injected and two patient-like metastatic models of HCCLM3-R and HCCLM3-G in nude mice were established using surgical orthotopic implantation from subcutaneous tumor tissues. Cell proliferation, karyotype, biomarker expression, tumor growth and metastasis of HCCLM3-R and HCCLM3-G were analyzed *in vitro* and *in vivo*.
Results: rfp and gfp genes were integrated in genomic DNA of HCCLM3. HCCLM3-R and HCCLM3-G expressed stable red and green fluorescence, stable and intense, 300 days after 60 consecutive passages, and also positively expressed CK8+, P16+, AFP+ and negatively expressed HBsAg-. And their biomarker expression and karyotype were found to be similar to those of the parental HCCLM3, and their tumorigenesis occurred in 10 nude mice without exception following a subcutaneous injection and did the same in 20 nude mice following an orthotopic implantation. The results showed that the rate of spontaneous metastasis to the liver and lung and peritoneal seeding was 100%, 100% and 90%, respectively.
Conclusions: Stable fluorescent protein-expressing HCCLM3-R and HCCLM3-G xenografts in nude mice could be of two useful models for studying mechanisms of HCC growth and metastasis in real time.

PP-147
Clinical applications of computerized tomography 3-D reconstruction imaging for diagnosis and surgery in children with large liver tumors or tumors at the hepatic hilum
Lu Hong ting[1], Dong Qian[1,1]
[1] *Qingdao University*
The study assessed the benefits of 3-D reconstruction of spiral CT scans for the diagnosis of and surgical guidance to large liver tumors or tumors at the hepatic hilum. We retrospectively analyzed 25 cases of children with such tumors treated in past 7 years.The patients were examined by 3-D reconstruction using 64 slice spiral CT. In 20 cases, the volume of tissue removed exceeded 1/3 the entire volume of the liver. In 5 cases, the excised tissue represented less than 1/3 of the total liver volume, but the location of the tumor was adjacent to major hepatic vessels. Pathological diagnoses included hepatoblastoma (n = 16), hepatocellular carcinoma (n = 2), mesenchymal hamartoma (n = 4), teratoma (n = 1) and adenoma (n = 2). All children had curative resections with tumor-free microscopic margins. 3-D CT imaging can provide high quality images and accurate location of the tumors. It could help the surgeon identify the tumor borders accurately and devise a safe surgical strategy. With its help the surgeon could identify vital hepatic blood vessels before operation, and can avoid massive hemorrhaging during operation.

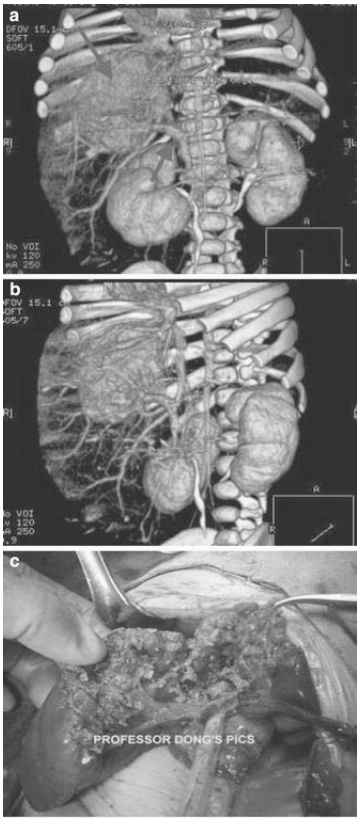

Fig.1 The image clearly illustrates the borders of the tumor and the location of tumor relative to hepatic blood vessels

PP-148

The effects of saikosaponin-d on the cell immune functionin in rats with hepatocarcinoma

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Objective: To study the effects of saikosaponin-d on T lymphocyte subsets in rats with hepatocarcinoma.

Methods: Ninty SD male rats were randomly divided into 5 groups: normal control group（10）, hepatocarcinogenesis model group (20 induced by low doses of diethylnitrosamine interruptedly), saikosaponin-d group with different doses (2.0、1.5、1.0 ㎎/㎏). Rats were administered with saikosaponin-d when they were induced by diethylnitrosamine for 14 weeks. All rats were killed in the 18th week, T lymphocyte subsets in peripheral blood from all rats were measured by flow cytometry.

Results: In the treatment with saikosaponin-d groups the level of CD_4^+, and CD_4^+/CD_8^+ was significantly increased compared with that in the model group, while the level of CD_8^+ was markedly decreased.($P < 0.05$).

Conclusion: Saikosaponin-d could improve the immune function in rats with hepatocarcinoma.

PP-149

Clinical applications of computerized tomography 3-D reconstruction imaging for diagnosis and surgery in children with large liver tumors or tumors at the hepatic hilum

Lu Hong Ting[1,1], Dong Qian[1]

[1] *Qing Dao University*

The study assessed the benefits of 3-D reconstruction of spiral CT scans for the diagnosis of and surgical guidance to large liver tumors or tumors at the hepatic hilum. We retrospectively analyzed 25 cases of children with such tumors treated in past 7 years.The patients were examined by 3-D reconstruction using 64 slice spiral CT. In 20 cases, the volume of tissue removed exceeded 1/3 the entire volume of the liver. In 5 cases, the excised tissue represented less than 1/3 of the total liver volume, but the location of the tumor was adjacent to major hepatic vessels. Pathological diagnoses included hepatoblastoma (n = 16), hepatocellular carcinoma (n = 2), mesenchymal hamartoma (n = 4), teratoma (n = 1) and adenoma (n = 2). All children had curative resections with tumor-free microscopic margins. 3-D CT imaging can provide high quality images and accurate location of the tumors. It could help the surgeon identify the tumor borders accurately and devise a safe surgical strategy. With its help the surgeon could identify vital hepatic blood vessels before operation, and can avoid massive hemorrhaging during operation.

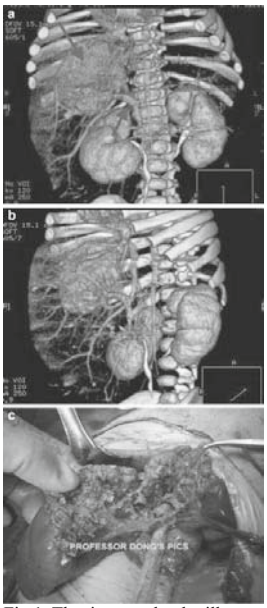

Fig.1 The image clearly illustrates the borders of the tumor and the location of tumor relative to hepatic blood vessels

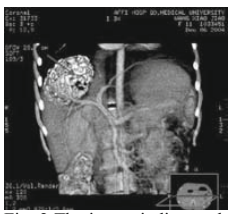

Fig. 2 The image indicates the size and location of the tumor and the location of the tumor relative to major blood vessels

PP-150

Computer-simulated three-dimensional thermal fields of implanted 915 MHz internal cooled-shaft microwave ablation: initial experimental study of ex vivo and in vivo porcine livers

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To explore the thermal distribution of a 915 MHz internal cooled-shaft microwave (MW) ablation by computer-modeled three dimensional in an equivalent MW phantom and in porcine livers.

The new-designed needle antenna used in present study could be easily punctured into the deep tissue guided by ultrasound imaging. Relying on the initial transient heating of the various spots pre-arranged in tissue-equivalent MW phantom, the mathematical expressions of specific absorption rate (SAR) induced by MW radiation were fitted under the three given outputs (60W600s, 70W600s, and 80W600s), respectively. Three-dimensional (3-D) temperature fields were mimicked with a finite-element model (FEM) basing on the Pennes' bio-heat transfer formula. Then the modeled thermal fields were validated and corrected with ablated experiments of ex vivo and in vivo porcine livers.

The actually measured temperature curves and the corresponding simulated ones good coincident tendency in 80.8% (59/73) and 61.3% (19/31) for ex vivo and in vivo ablations respectively. For in vivo experiments, rich blood flow perfusion of normal hepatic tissue was an important factor influencing the ablated lesions and the temperature rise.

Though some limitations occurred, the study demonstrated that computer-simulated 3-D temperature distribution was an accurate and reliable method in animal experimental study for prediction, evaluation and visualization of the thermal field induced by implanted 915 MHz internal cooled-shaft MW ablation. Further clinical applications of the ablative technique for hepatic tumors would be inspiring.

PP-151

Laser capture microdissection combined with proteome analysis in determining differential expression of DDAH -1 in hepatocellular carcinoma

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Objective: To investigate the differential expression of dimethylarginine dimethylaminohydrolase1 （ DDAH-1 ） in hepatocellular carcinoma（HCC）and surrounding non-tumor tissues using laser capture microdissection（LCM）combined with proteome analysis.

Methods: Forty surgical specimens of HCC were from patients who were treated in Eastern Hepatobiliary Surgery Hospital in 2003 -2006. LCM was applied to isolate hepatic parenchymal cells of cancerous tissues and surrounding non-cancerous tissues. Two-dimensional electrophoresis（2-DE）was used to screen for the differential expression proteins（the differential frequency >80% and the intensity >3 times）. The differentially expressed proteins were identified by electrospray ionization-tandem mass spectrometry（ESI-MS/MS）and matrix-assisted laser desorption/ionization time of flight mass spectrometry （MALDI-TOF-MS）. Western blotting and immunohistochemistry were used to analyze the differential expression of DDAH-1 in HCC.

Results: Totally 20 protein spots were identified to be differentially expressed between HCC tissue cells and the non-tumor tissue cells. Using ESI-MS/MS and MALDI-TOF MS technology，12 spots were identified by peptide mass finger print（PM F），with 5 spots upregulated and 7 spots downregulated in HCC cells，including metabolism related proteins，cell signal proteins，binding proteins and so on. Among these proteins，DDAH-1 is a critical regulator of the nitrogen monoxidum（NO）signaling pathway. Western blotting showed that the expression of DDAH-1 was markedly incresed in 16 of 20 HCC tissues. Immunohistochemistry showed that DDAH-1 was localized in the cytoplasm and was highly expressed in HCC tissues.

Conclusion: Twelve differentially expressed proteins have been identified in HCC cells. DDAH-1 is overexpressed in HCC tissue，which may play a very important role in the mechanism of hepatocarcinogenesis

PP-152

Enhanced B7-H4 expression is involved in the tumor cell proliferation of intrahepatic cholangiocarcinoma

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Intrahepatic cholangiocarcinoma (ICC) is a rare tumor usually associated with poor survival. Although recent imaging techniques have allowed for the early detection of this tumor, its pathogenic mechanisms remains unknown. B7-homologue 4 (B7-H4), a recently identified member of B7 superfamily, has been described to actively participate in inducing tumor cell immunoescape. Nevertheless, whether B7-H4 also takes part in the pathogenesis of ICC still unclear. We here investigated the expression and the functional activity of B7-H4 in human ICC *in vitro* and *in vivo*. Immunohistochemical analysis revealed significantly B7-H4 up-regulation on 28 neoplastic ICC whereas no expression could be detected on normal liver tissues. Followed up analyzed indicated that disease-free survival time was shorter in cases with B7-H4 expression than in those without B7-H4 expression ($P<0.05$; log-rank test). To investigate the potential mechanism of B7-H4 in tumor progression, the pattern of B7-H4 localization was compared with CD3, CD8, CD68, TUNEL and PCNA positive cells, and only the proportion of B7-H4 positive tumor cells was positively related to the number of PCNA–positive cells ($P<0.01$). Moreover, transfected B7-H4 to a cholangiocarcinoma cell line-KKU-100 promote these cell proliferation, whereas, siRNA-mediated knockdown of B7-H4 mRNA and protein expression in the other cholangiocarcinoma cell line-QBC939 cells decreased the cell proliferation. B7-H4 overexpression in a majority of ICC and functional activity in direct inducing tumor cell proliferation indicated it is a new target for therapeutic intervention.

PP-153

Decreased hepatitis B virus load after transcatheter arterial chemoembolization therapy for patients with HBV-related hepatocellular carcinoma

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[1] Liver cancer institute, [2] Fudan University

Objective: To evaluate relations between TACE and HBV reactivation.

Methods: From 2001 to2007, 54 patients with HBV-related HCC were studied retrospectively. All the patients were treated with at least one session of TACE using 5-fluorouracil (0.5-1.0g), cisplatin (20-80mg), mitomycin (0-20mg) and lipiodol (0-20ml). HBVDNA levels of pre-treat and post-treat were measured by PCR. HBVDNA$\geq 1\times10^3$ copy/ml was recognized positive.The binary repeated measured data was statistically analyzed by Chi-Square tests.

Results: HBVDNA positive ratio was decreased after TACE (68.52% vs 31.48%，$p<0.0001$), the difference between the two rates was statistically significant.

Conclusion: Rather than aggrevate HBV reactivation, TACE can inhibit HBV reactivation in HCC patients carrying HBV.

PP-154

Extracellular domain of human 4-1BBL enhances the lysis of hepatocarcinoma cells mediated by dendritic cells and cytotoxic T lymphocytes

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[1] *Tianjin Third Central Hospital,* [2] *Tianjin Key Laboratory of Artificial Cells,* [3] *Institute of Hematology, Chinese Academy of Medical Science*

Objective: To study whether extracellular domain of 4-1BBL (ex4-1BBL) can enhance the lysis of human hepatocarcinoma cell line SMMC-7721 mediated by dendritic cells (DC) and cytotoxic T lymphocytes (CTL)**.**

Methods: The soluable extracellular portion of human 4-1BBL was expressed in a prokaryotic host and purified by a one-step affinity chromatography. DCs induced from human cord blood were sensitized with lysate antigen of SMMC-7721. Then autologous lymphocytes and DCs were co-cultured with or without ex4-1BBL. ELISA was used to determine IL-2 and IFNγ concentrations of the culture supernatant. Lymphocyte viability was measured by a nonradioactived LDH test. To evaluate the cytotoxicity of CTL, lymphocytes were primed by unsensitized DCs and sensitized DCs with or without ex4-1BBL. The susceptibility of the hepatocarcinoma cell to specific lysis in the presence of ex4-1BBL was explored with Cyto-Tox 96 assay kit.

Results: Ex4-1BBL increased the levels of IL-2 and IFNγ secreted by CTL with a dramatic increase at 48h and 72h cultivation and show a decreased trend of the LDH in the supernatant, suggesting the improved status of activated cells. Cord blood derived-DCs could activate T cells and resulted in an inhibitory effect on the growth of SMMC-7721 with a killing rate of (43.5±1.50) %. Further, the additional ex4-1BBL strengthened the cytotoxic efficacy and the killing rate was up to (52.16%±4.84) %.

Conclusion: Ex4-1BBL can improve the cytotoxic effects on tumor cells in vitro and this might associated with the increased cell expansion, up-regulated cytokine secretion and improved activation of CTL.

PP-155

Mechanism-based combinational therapy of hepatoma with artemisinin compounds and gemcitabine: In vitro and in vivo activities and mechanisms of action.

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[1] *Institute for Nutritional Sciences, Shanghai Institute for Biological Sciences*

Hepatocellular carcinoma (HCC), one of the leading causes of cancer-related death worldwide, especially in China and Africa, has a low response rate and poor outcome after conventional therapy. There is a pressing need to identify alternative therapeutic strategies for this disease. Artemisinin, a natural product antimalarial drug isolated from the plant *Artemesia annua L*, and some derivatives have recently shown antitumor activities, although the underlying mechanisms are not fully understood. In this presentation, we report a comprehensive, systemic comparison of the antitumor activities and molecular mechanisms among artemisinin and analogs (dihydroartemisinin, artemether, and artesunate) with *in vitro* and *in vivo* HCC models. Artemisinin and dihydroartemisinin exerted the greatest cytotoxicity to human hepatoma cells, but much lesser effects on normal liver cells. They inhibited cell proliferation and induced apoptosis in hepatoma cells with various p53 statuses, HepG2 (p53wt), Huh-7 and BEL-7404 (p53mt) and Hep3B (p53null). Their anti-proliferative effects were associated with G1 phase arrest and with decreased levels of cyclin D1, cyclin E, cyclin-dependent kinase (Cdk) 2, Cdk4, and E2F1 and increased levels of p21 and p27 and their effects on apoptosis were associated with caspase 3 activation, increased Bax/Bcl-2 ratio, MDM2 down-regulation, and increased PARP cleavage and Rb levels. They also inhibited tumor growth in mice bearing HepG2 xenograft tumors,

significantly increasing the chemotherapeutic efficacy of gemcitabine and confirming *in vivo* effects on gene expression observed *in vitro*. In conclusion, the artemisinin compounds are promising candidate therapeutics for HCC used alone or in combination with conventional therapeutic agents.

PP-156

Validation of MELD-based model for prediction of survival in Korean patients with hepatocellular carcinoma (HCC)

Kyung Woo Park[1], W Ray Kim[2], Rachel A Pedersen[2], Byung Ho Nam[1], Joong-Won Park[1]

[1] National Cancer Center, Goyang, Korea, [2] Mayo Clinic, MN, USA

Background & Aims: Previously, we developed a new survival prediction model for HCC using the Model of End stage Liver Disease (MELD) as the gauge of liver dysfunction in Korean patients (Kim, AASLD 2006). The aim of current study is to investigate the performance of this model in new cohort of Korean HCC patients.

Methods: Between 2004 and 2005, 819 HCC patients were enrolled and followed by December 2006 at National Cancer Center Hospital, Korea. The performance of MELD-based model was analyzed using the proportional hazards regression analysis and C-statistics as described previously.

Results: The mean age of the patients was 57 years. According to modified UICC stage, stage I, II, III, IVa and IVb was 9.3%, 25.0%, 22.0%, 24.1% and 19.6%, respectively. The mean of MELD score was 8.4±3.3. Overall 3-year survival rate was 33.6% (median 16.2 months). In multivariate analysis, age (HR 0.86, 95% C.I. 0.78-0.94), number of tumor (HR 1.23, 95%C.I. 1.14-1.31), tumor size (HR 1.21, 95% C.I. 1.11-1.32), portal vein invasion (HR 1.71, 95% C.I. 1.32-2.22), metastasis (HR 1.76, 95% C.I. 1.38-2.25) were significant predictable factors. The MELD-based model included following variables: age in decades, hepatitis B virus in etiology, number of tumor, size of the largest nodule, portal vein invasion, extrahepatic metastasis, serum alpha-fetoprotein and albumin level. The C-statistics of this model in new cohort was 0.775 (95% C.I. 0.753-0.797).

Conclusion: The MELD-based model shows good performance in new cohort and may be useful for prediction of survival in patients with HCC.

PP-157

Metabolic profiling of bile in Egyptian patients with cholangiocarcinoma: insights into pathogenesis and regional variation

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Background: Recent epidemiological studies have shown that incidence rates for CCA are increasing worldwide, with substantial regional variation.

Aim: To establish a metabolic profile of bile in Egyptian patients with CCA, compared to non-Egyptian patients using *in vitro* magnetic resonance spectroscopy (MRS) at 11.7T for identification of potential early disease biomarkers.

Methods: Eight CCA bile samples and 21 bile samples from disease controls with benign biliary diseases were analysed using ^{1}H and ^{31}P MRS (JEOL ECP+ 500). Bile spectra were analysed using KnowItAll® Informatics system software.

Results: Phosphatidylcholine (PtC) concentrations were significantly reduced in all CCA patients, compared to disease controls. However, Egyptian CCA patients had significantly lower biliary PtC levels, compared to non-Egyptian patients. These changes were also evident in patients whose cholestasis had been relieved by previous biliary stenting.

Conclusion: Reduced biliary PtC may lead to increased concentration of toxic bile acids through impaired micelle formation, which could lead to bile duct inflammation, a risk factor for cancerous change in the biliary epithelium. Reduced bile PtC in Egyptian patients compared to non-Egyptian patients suggests that genetic variation in biliary transporters, such as MDR3 (PtC transporter), may increase susceptibility to CCA. Such differences may partly explain the global variation in CCA incidence. MRS of bile may be a useful future biomarker for the diagnosis of biliary tract cancer and could potentially inform the direction of future genetic studies on disease susceptibility in different populations.

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The effect of a mistletoe preparation in unresectable hepatocellular carcinoma, open labeled, multi-centric study

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In Egypt Hepatocellular Carcinoma (HCC) reached up to 6%. Although a wide range of therapeutic options are available, very few cases can be cured. Immunomodulation by Mistletoe has been investigated in several studies. The aim of this study was to evaluate the effect of a Mistletoe preparation (an aqueous injectable solution in the form of ampoules) as regard efficacy, safety and disease related symptoms in unresectable HCC. This open labeled, multi-centric study included 60 patients with pathologically proved, measurable, unresectable HCC. Each patient received 2 ampoules subcutaneous weekly. Each ampoule contains one-milliliter (15 mg extract of 20 mg mistletoe herb) that is equivalent to 10000 ng Lectins/ml. According to the WHO response criteria and as judged by conventional CT scan, among 51 eligible patients, overall tumor response was 25.5% (13/51) , 5/51 (9.8%) showed complete response, 8/51 (15.7%) showed partial response, in 29/51 (56.5%) of patients the disease was stationary and 9/51 (17.6%) showed disease progression. At 24 months the overall cumulative survival was 39.5%. Drug related fever and erthyma at the site of injection was reported by 85% of patients. No major side effects or toxicity were encountered. Disease related symptoms such as pain, performance status (WHO) and appetite were subjectively improved. Subcutaneous Mistletoe preparation may be a promising method of treatment in unresectable HCC. Large randomized controlled studies are needed.

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Extrahepatic malignant biliary obstruction: correlation between imaging findings, tumor markers and intraductal biopsies in diagnosis

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The distinction between malignant and benign biliary strictures remains difficult. PURPOSE: The purpose of this study was to evaluate the accuracy of ERCP, cholangioscopy, intraductal biopsy, endoscopic US, CT abdomen, intraductal US and tumor markers in the diagnosis of biliary strictures in patients affected by extrahepatic malignant obstruction.

Subjects and methods: Patients admitted at the Hepatology Department, National Liver Institute, Menoufiya University and Medicine 1 department, Faculty of Medicine, Erlangen University, for emergency or elective ERCP presented with biliary stricture and/or obstruction and suspected for malignant lesions, were included in this study. All patients were subjected to: thorough medical history taking, liver tests, serum tumor markers (CEA, CA-19-9), abdominal US, spiral CT scan of abdomen, endoscopic US, ERCP, cholangioscopy and intraductal biopsies. Results revealed that ERCP, cholangioscopy, intraductal biopsy, endoscopic US, CT abdomen, intraductal US and tumor markers revealed sensitivity 84.5, 92.8, 73.3, 76.9, 74.2, 83.3, 57.7 % respectively; specificity 63.8, 100, 100, 85.7, 46.8, 75, 48.5% respectively; positive predictive value 88.6, 75, 46.6, 96.7, 87.2, 90.6 and 70.6% and negative predictive value 63, 100, 100, 40, 86.2, 60 and 36.1% respectively. CONCLUSIONS: Abdominal ultrasounds almost always identified the cause of stenosis and suggested its neoplastic nature if it exhibited a mass-like appearance (extraductal or growing into the choledochus). On the other hand, lesions with parietal thickening, particularly if smaller than 1 cm, require intraductal biopsy because of the high risk of unnecessary procedures for benign lesions.

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Hepatitis B virus X protein interaction with p53 and down-regulating PTEN expression relates to its anti-apoptosis role

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Objective: To investigate whether hepatitis B virus X protein (HBx) blocks adrimycin-induced apoptosis of hepatocellular carcinoma cells by regulating p53 and PTEN pathway.

Methods: $HepG_2$, $HepG_2$/GFP, and $HepG_2$/GFP-HBx cells expressing GFP-HBx fusion protein were treated with adriamycin (2.5 μg / ml). Apoptotic cell death was determined in adriamycin-treated cells by observing morphologic changes, trypan blue exclusion, and flow cytometry analysis. Meanwhile, expression of p53 and PTEN were determined by RT-PCR analysis and SDS-PAGE electrophoresis. Binding of HBx to p53 protein was assay by co-immunoprecipitation and SDS-PAGE electrophoresis.

Results: Significant apoptotic cell death in HepG2, HepG2/GFP cells appeared as observed cells becoming rounded, shrank, and detached respectively after treatment with adirmycin while no significant cell death was observed in HepG2/GFP-HBx cells. Flow cytometry analysis showed that apoptosis rates in $HepG_2$/GFP-HBx (3.94%) was significant lower than that in $HepG_2$ (59.03%) and $HepG_2$/GFP cells (61.38%) at 36 hours after treatment with adriamycin ($P<0.001$). In comparison with HepG2, HepG2/GFP cell, levels of PTEN mRNA and PTEN protein decreased in $HepG_2$/GFP-HBx cell while no changed levels of p53 mRNA and p53 protein were observed. Co-IP analysis showed that HBx protein directly bound to p53.

Conclusion: HBx interacts with p53, and suppresses expression of PTEN while HBx blocks adriamycin-induced HepG2 cell apoptosis. It suggests that HBx blocks chemotherapeutic drug-induced apoptosis of hepatocellular carcinoma cell involving interaction with p53 and down-regulating PTEN.

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Prognosis and treatment strategies on synchronous colorectal liver metastases in Chinese patients

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Objectives: To investigate prognostic factors and treatment strategies on synchronous colorectal liver metastases (CLM) patients in China.

Patients and Methods: It was a retrospective study conducted in 129 patients diagnosed with synchronous CLM from January 2003 to December 2006 in Zhong Shan Hospital, Fu Dan University. Both Kaplan-Meier and Cox regression model were used for survival and prognostic analyses.

Results: The median overall **s**urvival time (MOS) of these 129 patients synchronous CLM patients was 19 months after followed up for a median time of 36 months. High level of CEA at onset could be an independent poor prognostic factor for CLM patients with the relative risk of 2.328 (95% confidence interval: 1.017~5.325). First-line therapy with one stage of primary cancer and liver metastases resected would have a longer MOS of 26 months. Different options in first-line therapy with primary cancer resection, interventional treatment and systemic chemotherapy had MOS of 21 months, 10 months and 14 months, separately. Two patients who received best supportive care (BSC) only had MOS of 3 months. MOS increased with the number of active chemotherapeutic agents used (46 months for three agents, 15 months for two agents and 13 months for one agent, P=0.006).

Conclusions: For synchronous CLM patients, high level of CEA at onset suggested be a poor prognostic factor. One stage resection of primary sites and liver metastases may offer a rather long survival. More effective application of systemic chemotherapy is strongly recommended in Chinese patients.

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Unresectable extrahepatic malignant biliary obstruction: drainage with plastic or metal endoprosthesis? A prospective randomized controlled trial

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Palliative biliary drainage is a critical step in improving life style of patients with unresectable extrahepatic malignant biliary obstruction. Drainage may be surgical, percutaneous or endoscopic. Endoscopic approach is preferred as it is less invasive and carries better life quality for the patients. Endoscopic approach is carried out through either plastic stent or metal stents insertion. Aim of the study was to compare the stent patency, complications and patient survival between patients who received either metal or plastic stents. Subjects and methods: 50 patients aged (66±8.5 years) with unresectable malignant biliary obstruction and life expectancy more than 3 months were randomized to receive either plastic stents (25 patients, group I) or metal stents (25 patients, group II). Results: Initial drainage was achieved in all patients with metal stent group 25 from 25 patients (100%), while initial drainage was achieved only in 24 patients from 25 (96%) in plastic stent group. The patency (mean and standard deviation) in plastic stent was (277.90 ± 74.00 days) while it was (413.74 ± 38.79) with significant difference (p value 0.02 (<0.05). Survival was higher in metal stent group (418.75 ± 45.36 days) versus (309.12 ± 20.00) but without statistical significance difference (p value= 0.26) (>0.05). Conclusions and recommendations: metal stent insertion is recommended in palliative drainage of biliary obstruction in patients with unresectable malignant biliary obstruction with life expectancy more than 3 months.

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Feasibility and efficacy of dose-reduced transcatheter arterial chemoembolization (TACE) for primary liver cancer patients with portal vein thrombosis

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Purpose: To evaluate the efficacy and safety of does-reduced transcatheter arterial chemoembolization (TACE) for primary liver cancer patients with major portal vein thrombosis.

Methods: 37 patients with major portal vein and/or its first branch thrombosis underwent does-reduced TACE were retrospectively analyzed. The Child's classes were A for 34 patients and B for 3.

Results: 14 patients achieved good precipitation of lipiodol in the portal vein thrombosis, the other 23 patients with poor precipitation of lipiodol in portal vein thrombosis. The overall median survival time was 11.7 months. The cumulative survival rates were 75.0% (6 months), 41.0% (12 months), and 17.6% (18 months). Patients with well precipitation of lipiodol, the median survival time was significantly longer than patients with poor lipiodol precipitation (16.2 VS 6.8 months, $p<0.05$). The 6m, 12m and 18m survival rate of patients with well lipiodol precipitation was significantly better than that of with poor lipiodol precipitation (100%、 63.5%、31.8% VS 60.3%、13.0%、0%，$P<0.05$). Multi-variable analysis shows that the extent of lipiodol precipitation was the only significant predicting factor for efficacy of therapy (Cox regressive coefficient 1.67, RR 5.31). All patients were safely tolerated with dose-reduced TACE except 1 died of liver failure 2 weeks after TACE, 1 case of uppr gastrointestinal bleeding, the other 2 with increased Child-pugh scale.

Conclusions: Dose-reduced chemoembolization for primary liver cancer patients with major portal vein thrombosis is safe, and can partially achieve curative effect especially for patients with good precipitation of lipiodol in the portal vein thrombosis.

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Comparison of risk factors of HCC developed in cirrhotic or non-cirrhotic liver.

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The aim of this study was to compare the clinical and biological characteristics of patients with HCC developed in cirrhotic (CL) or non cirrhotic liver (NCL)

Material and methods: From 2005 to 2007, 301 patients were referred to our unit for HCC. In 48 cases, HCC was developed in NCL (F0-F3). For each patient, following data were recorded: age, sex, alcohol and tobacco consumption, BMI, underlying disease as diabetes, arterial hypertension or dyslipidaemia, virus B and C status, glycaemia, cholesterolaemia, triglyceridemia.

Results: 48 patients (male 89%) without cirrhosis were compared to 253 cirrhotic patients (male 85%). Patients are older in the group of NCL (71 vs 66 years p= 0,0025). Consumption of alcohol (80 g/day vs 25 p=0,0001) and tobacco (22 cigarettes/day v s 10 p= 0,05) was higher in cirrhotic patients. Hepatitis C (29% vs 14% p=0,08) and hepatitis B (9% vs 2% p= 0,049) were more frequent in cirrhotic patients. There was no difference for BMI (25 vs 24), diabetes (23% vs 18%). Arterial hypertension was more frequent in the cirrhotic group (39 vs 32% p=0,0002) contrary to dislipidaemia (13,5% vs 28,5 p=0,003)

Cholesterolemia was higher in NCL patients (5,08 vs 4,44 mmol/l p = 0,01), triglyceridemia (1,21 vs 0,98 mmol/l) and glycaemia (0,85 vs 1,1 g/l) did not differ.

Conclusion: HCC in non-cirrhotic liver is a specific entity. Risks factors remain unclear but differ from those observed in cirrhotic patients. Indeed, large epidemiological studies are necessary to a better understanding of this pathology.

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Meta-analysis of postoperative transcatheter arterial chemoembolization in preventing relapses after curative liver resection for hepatocellula carcinoma

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Background: Transcatheter hepatic arterial chemoembolization (TACE) has been applied on the management of liver malignancies for a long time. However, the reports of efficacy of postoperative TACE in preventing recurrence after curative resection of hepatocellular carcinoma (HCC), is conflicting.

Methods: Literatures of trials testing radical resection of HCC plus postoperative TACE against surgery alone, were collected from MEDLINE (1966-2007), EMBASE (1984-2007), The Cochrane Library database and Chinese Biomedicine Collaboration (1980-2007) in a systematic review, the studies were evaluated by two independent investigators following the Cochrane Collaboration guideline and the extracted data was analyzed using Stata7.0 software.

Results: Six prospective studies which included 430 HCC patients met the inclusion criteria and were subjected to meta-analysis.The 1,3and5-year recurrence rates among patients receiving operation plus postoperative TACE were significantly (P≤0.002) lower than those received operation alone (OR is 0.32(0.17, 0.60), 0.34(0.0.21, 0.55) and 0.47(0.29, 0.77) for 1, 3 and 5-year recurrence rate, respectively).

Conclusion: This meta-analysis suggests that curative resection plus postoperative TACE is superior to operation alone in preventing HCC recurrence.

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Effect of hepatic blood inflow occlusion without hemihepatic artery control during hepatic resection in HCC patients

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Objective: To investigate the effects of hepatic blood inflow occlusion without opposite hemihepatic artery control (modified Pringle maneuver) for liver resection in HCC patients.

Method: 59 HCC patients undergoing major liver resection were randomly allocated to the modified Pringle maneuver group(MP group,19 cases), Hemiliver inflow occlusion group(H group,20 cases) and Pringle maneuver group(P group,20cases). Clinical parmeters included operation time, blood loss and the postoperative course of these groups were compared.

Result: The operation times was significantly shorter in MP and P group than in H group, and serum ALT and AST levels at 1st postoperative day in MP and H groups were significantly lower than in P group. Blood loss during operation, recovering time of liver function, serious complications and hospital stay time did not differ significantly between three groups. There was no death in any group.

Conclusion: Hepatic blood inflow occlusion without opposite hemihepatic artery control (modified Pringle maneuver)is equally effective in bleeding control compared with Pringle maneuver and Hemiliver inflow occlusion techniques, but simple, safe and less injury.

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Identification of proteins associated with tumor recurrence after liver transplantation for hepatocellular carcinoma, through laser capture microdissection coupled with cICAT-2D-LC-MS/MS

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Dou-Sheng Bai and Zhi Dai contributed equally to this abstract.

The purpose of this study was to identify the proteins that play a pivotal role in metastasis and recurrence of HCC after LT. We applied a cleavable isotope-coded affinity tag (cICAT) technology to quantitate relative changes in protein levels between the recurrence and DFS groups. We identified a total of 149 proteins with two-dimensional LC coupled with tandem MS (2D-LC-MS/MS), including 52 differentially expressed proteins (twofold or higher changes) in the recurrence compared to the DFS group. The down-regulation of plastin 3 and the up-regulation of thioredoxin like 1 and calpain small subunit 1 (Capn4) were confirmed by real-time PCR, immunohistochemistry and Western blotting. Capn4, a protein with relevant interactions with many migration–invasion-related proteins, has attracted more attention. The small-interfering-RNA-mediated knockdown expression of Capn4 in higher metastatic HCC cell lines significantly inhibited its invasive ability. Our study suggests that quantitative proteomics may provide a rich opportunity to investigate recurrence-related proteome of HCC after LT, and these proteins may serve as new prognostic biomarkers and potential therapeutic targets.

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Hepatocellular carcinoma presenting with bone metastasis: Clinical characteristics and prognostic factors

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Purpose: The survival of patients with hepatocellular carcinoma (HCC) has been prolonged with improvements in various diagnostic tools and treatment modalities. Consequently, bone metastases from HCC are diagnosed more frequently. We investigated the clinical features, prognosis, treatment outcomes, and prognostic factors of HCC presenting with bone metastasis.

Methods: Between June 2000 and April 2007, we recruited 37 consecutive HCC patients presenting with bone metastasis. These patients were divided into an untreated control group (n=16) and a treated group (n=21).

Results: The mean age of the patients was 61.1 years (male:female, 31:6). The most common cause of HCC was hepatitis B virus infection (56.8%). Twenty-two patients (59.6%) were Child-Pugh class A and 15 (40.5%) were Child-Pugh class B. Spinal metastasis was most common and noted in 21 patients (56.7%). The treatment modalities in the treated group included intra-arterial chemotherapy in nine patients (42.8%), systemic chemotherapy in five (23.8%), and both intra-arterial and systemic chemotherapy in seven (33.4%). The median survival of all patients was 6.2 months (range, 0.7–46.6); that of untreated control group and the treated group was 2.6 (range, 0.7–42.2) and 9.7 (range, 0.9–46.6) months, respectively, with no significant difference (log-rank test, P=0.081). Cox regression analysis revealed that the presence of ascites at the initial presentation was the only prognostic factor (P=0.016).

Conclusion: Although our study showed that locoregional and/or systemic chemotherapy did not offer significant survival prolongation compared to supportive care in patients with HCC initially accompanied by bone metastasis, a more large-scaled randomized study might be required.

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Imaging guided standardized treatment for hepatocellular carcinoma with radiofrequency ablation

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Objectives: To investigate the role of imaging guided standardized RFA and summary its clinical outcome.

Method: A total of 350 patients with 476 HCC underwent RFA in our institute. Among them, 297 patients (84.8%) were not candidate for surgery; 265 patients (75.7%) had advanced HCC or recurrent HCC after surgery. 248 patients with 284 >3.5cm HCC were treated with mathematical optimal protocol (group A); 81 patients with 115 HCC were treated with instruction of CEUS (group B). 71 patients with 74 hypervascular HCC were treated with new techniques (group C). Individual protocols and strategies were developed for peripherally located HCC (group D).

Result: The tumor necrosis rate were 90.5 for group A, 92.2% for group B, 89.2% for group C, 92.6% for group D, respectively. The 1, 3, 5 year survival for these 350 HCC patients were 87.9%, 58.7% and 43.2%, respectively. The major complications rate was 2.3% (12/532 sessions).

Conclusions: The standardized treatment of RFA based on imaging information can effectively improve RFA efficacy and safety in HCC.

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Hepatocellular carcinoma invading main portal vein: Treatment with tanscatheter arterial chemoembolization and portal vein stenting

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Purpose: We performed a retrospective analysis of the safety and efficacy of 58 patients with hepatocellular carcinoma invading main portal vein treated by percutaneous transhapatic portal vein stenting (PTPVS) and transcatheter arterial chemoembolization (TACE).

Materials and Methods: A total of 58 procedures of PTPVS were performed. TACE was undertaken to control hepatocellular carcinoma after PTPVS. The clinical effects, complication, digital substraction angiographic appearance, stent patency rates, cumulative survival rates and predictive factors for survival were evaluated. The Kaplan-meyer method and log rank test were used for survival analysis. Multivariable analysis was also conducted using Cox proportional hazards model.

Results: No patient died during stent placement or within the first 24 hours. No severe procedure-related complications were observed. After stent placement, the mean portal venous pressure levels decreased from 41.43 ± 8.56 cmH2O to 37.19 ± 7.89 cmH2O ($p<0.01$). At the time of analysis, 9 of 58 patients were alive. The 60-, 180-, 360-, 720-day cumulative patency rates were 98.1%, 71.0%, 52.6% and 42.1%, respectively, with a mean patency time of 552.9 ± 88.2 days and a median patency time of 639 days. The 60-, 180-, 360-, 720-day cumulative survival rates for the total study population were 89.7%, 39.7%, 16.9% and 14.1%, respectively, with a median survival of 139 days.

Conclusion: Treatment with PTPVS-TACE for hepatocellular carcinoma invading main portal vein is a safe and effective treatment modality.

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Estrogen and its receptors impact on liver metastasis cancer from colorectal carcinoma

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Objective: we assay 1) the expression status of estrogen receptors (ER), including two subtypes: ERα and ERβin metastatic liver carcinoma tissues from colorectal carcinoma, the original cancer focus and normal colorectal mucosa nearby; and 2) the serum estrogen level in metastatic liver carcinoma patients.

Method: Immunohistochemical stainning with antibody against ERαand ERβprotein was performed on histological sections respectively, whose samples derived from metastatic liver carcinoma, colorectal cancer mucosa, normal mucosa distant from cancer tissue and prepared from paraffin block. Estrogen RIA Kit was used to assay serum estrogen level, whose samples came from patients with liver metastasis and without metastasis.

Result: Metastatic liver carcinoma expressed ERβ, and ERβexpression rate in liver metastasis, colorectal cancer mucosa and normal mucosa was 11.5%, 32.8%, and 53.8% respectively. There were significant differences among three tissues above in ERβexpression rate, P<0.05. ERαwas not seen in any sections. Serum estrogen levels in patients with liver metastasis and without metastasis were 103.44±54、164.23±68（pg/ml）respectively, and there was significant difference between two groups patients in statistic.

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Viral, genetic, and environmental risk factors for hepatocellular carcinoma (HCC) in Egypt

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The high incidence of HCC in Egypt has been attributed to the population prevalence of HCV (18%), but genetic and environmental risk factors were not studied in depth. Since 1999 we have been conducting a study to elucidate these associations. Cases with HCC (N=541) were recruited at the National Cancer Institute (NCI) of Cairo University. Controls (N=709) were non-cancer subjects matched by age, sex, and region, recruited from the orthopedic department of the same university. Subjects were interviewed about their lifestyle habits and occupational histories. Virological markers included anti-HCV, HCV RNA, HBcAb, and HBsAg. PCR generated genotypic data for xenobiotic metabolism genes, including CYP1A1, CYP2D6, GSTM1, GSTT1, COMT, PON1, EPHX, and NAT2. Multivariate logistic regression models yielded adjusted odds ratios (OR) and 95% confidence intervals (CI). We observed a strong association between HCC and HCV-RNA in serum (OR=11.0, 95% CI 7.5-16.2), and less so for current HBV infection (OR=5.7, 95% CI 2.8-11.6). Dual current infections had highly elevated risk (OR=43.1). Potential environmental associations included agricultural pesticides used by rural males, especially organophosphorus (OR= 2.7, 95% CI 1.3-5.3) and carbamate chemicals (OR= 2.9, 1.4-5.8). Modest associations were observed for aberrant genotypes of EPHX (slow metabolizer: OR=1.5, 95% CI 1.1-2.2) and CYP2D6 (extensive metabolizers: OR: 1.8, 95% CI 1.1-3.0), and these associations were further strengthened if the subjects reported tobacco smoking or pesticide exposures. In summary, these results confirm the predominance of HCV as the major risk factor for HCV in Egypt, with additional risk contributed by agricultural pesticides and genetic susceptibility.

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Up-regulations of TGF-B1 and IGF-II expression by hepatitis B virus replication in human hepatoma tissues

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Objective: To investigate the relationship between hepatitis B Virus (HBV) replication and the expressions of transforming growth factor (TGF)-β1 and insulin-like growth factor-II (IGF-II) in human hepatocellular carcinoma (HCC).

Methods: Liver HBV-DNA was detected by *in situ* molecular hybridization technique, and the expressions of TGF-β1 and IGF-II was detected by the immunohistochemistry in HCCs and their self-control non- cancerous tissues. The relationship was investigated between TGF-β1 or IGF-II expression and HBV replication or their clinical pathological characteristics. **Results:** The stronger expressions (83.3%) of TGF-β1 and IGF-II were found in HCC tissues. A significant difference was presented between in HCC tissue and in non-cancerous liver tissues ($P<0.01$). The positive expression of TGF-β1 in HCC was correlated to tumor differentiation, but neither to tumor size nor numbers ($P>0.05$). The levels of TGF-β1 and IGF-II expression were significantly associated with HBV replication with higher HBV-DNA-positive HCC than that in HBV-DNA- negative group. **Conclusion:** TGF-β1 and IGF-II in HCC tissues are overexpressed and associated withhepatic HBV replication and differentiation degree of HCC.

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Association between the nucleotide excision repair (NER) gene polymorphisms and genetic susceptibility of hepatocellular carcinoma

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* This research supported by Chinese High-Tech Program grants 2001AA224011 and 2002BA711A10.

Objectives: To evaluated the association between Nucleotide excision repair (NER) gene polymorphisms and risk of hepatocellular carcinoma (HCC).

Methods: A total of 434 HCC cases and 480 controls were recruited in Guangxi Province, China. Polymorphisms of ERCC1, XPA, XPC, XPD, XPF and XPG gene were genotyped by PCR-RFLP analysis.

Results: Stratified analysis by HBsAg status, no significantly association was observed between each genotype of 11 polymorphisms and risk of HCC after adjusting for gender, age, tobacco use status and pack-year, and alcohol use status, and correcting by Bonferroni Correction meanwhile. In the HBsAg(+) status, the genotype-environmental factors interaction were significant in 8 polymorphisms, respectively, and showed markedly association with HCC risk. In the HBsAg(-) status, however,only one genotype-age interaction was significant. A dose-dependent association between increasing number of the NER risk genotype and HCC risk was observed.

Conclusions: The NER gene polymorphisms can modify individual susceptibility to HCC.

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The mechanism study of targeted therapy for overexpressed PEG10

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Objective: To investigate the expression of the human liver cell line L02 PEG10 with drug sensitivity of human hepatocellular carcinoma cells. Adenovirus vector targeting and treatment.
Methods: LO2-PEG10 cell clone experiment with doxorubicin, cisplatin, 5-fluorouracil and adenovirus vector MDA-7 experimental medication. MTT, Flow cytometry and transplanted tumors in nude mice model PEG10 hepatoma cell resistance to the gene.
Results: The L02 cell lines stably expressing PEG10 for adding epirubicin, cisplatin and 5-fluorouracil, no apoptotic cells compared with control group (P <0.05); MDA-7 join the adenovirus vector, Apoptosis in the experimental group were significantly lower than those in the control group (P <0.05). Subcutaneous xenograft model in nude mice, MDA-7 can inhibit the growth of tumors.
Conclusions: PEG10 gene expression levels were significantly elevated in the human liver cell line. chemotherapeutic drugs for the treatment of hepatocellular carcinoma may not sensitive factor; MDA-7 adenovirus vector PEG10 gene expression in the liver can promote apoptosis and inhibit tumor growth. Targeting System for the treatment of liver cancer and the molecular mechanism for an experimental basis and theoretical basis.

PP-176
Long-term interferon therapy in patients with refractory chronic hepatitis C: Effect on histological liver damage and hepatocellular carcinogenesis
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The effect of interferon (IFN) therapy in preventing liver damage progression and hepatocellular carcinoma (HCC) in patients with non-sustained virological response (NSVR) is not determined. This study aimed to elucidate the role of long-term IFN therapy on the course of chronic hepatitis C (CHC) and the occurrence of HCC in refractory CHC patients compared with natural disease progression. This retrospective study enrolled CHC patients followed for a minimum of 10 (mean 15) years at a liver centre in Italy. The treatment group consisted of 100 patients who had not shown a sustained virologic and biochemical response after a mean duration of 48 months of IFN monotherapy. The control group included 72 patients who had not received any antiviral therapy because of individual contraindications or refusal.
Results: All patients were hepatitis C virus (HCV)-RNA positive. Baseline biopsy documented liver compensated cirrhosis in more control than treated patients (58% vs 37%). Overall, incidence of hepatocellular carcinogenesis was 48%. The highest incidence of HCC was observed in the control group [odds ratio (OR) 0.43; 95% confidence interval (CI) 0.23–0.80; $P < 0.01$), males, HBV/HCV coinfected patients and in patients with cirrhosis. According to Cox regression analysis, treated patients had a lower risk of developing cirrhosis [hazard ratio (HR) 0.51; CI 0.30-0.93; $P < 0.05$) and HCC (HR 0.54; 95% CI 0.30–0.97; P < 0.05) than controls. Our results suggest that long-term and high-dose IFN therapy might be considered a preventive approach to reduce or at least delay HCC occurrence in refractory CHC patients if administered before cirrhosis onset.

PP-177
A report on study of the treatment of the great HCC of octogenarian with Xiaoaiping injection, Jinlong caps and Octretide Acetate
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Objective: Introduce a new discovery in cancer therapy versus a clinicial study on the treatment of great HCC (Hepatocellular Cancinoma) of octogenarian with Xiaoaiping injection, Jinlong caps and Octretide Acetate.
Metheds: 2 patients with great HCC,one was a 80-year-old man, his AFP 785.49μg /L,there was a tumor (about 7.8*8.8cm) located in the right Hepatic lobe (Ultrasonography), and QOL (Quality of Life) 36. Another was a 76-year-old man, AFP 1393.40μg /L,there were several masses in the liver,the biggest one was about 5.88*7.39cm (Ultrasonography) ,QOL 34.
Therapy: The first patient was given Xiaoaiping 60ml,ivgtt,qd and Octretide Acetate 15-18μg /h,Civ for 16 days.The second patient received the same treatment but adding Jinlong caps 4 caps,po,tid at the same time.According to the result of imageology,change of AFP and QOL before and after the treatment,the therapeutic effect was determined.
Results: AFP of first patient reduced to 162.8μg /L,the tumor 7.9*10.2cm (enlarged by 18%) ,QOL 48,the effect is SD.Second AFP reduced to 1107μg /L,the tumor reduced to 4.51*4.33cm(reduced by 70%), QOL 53, the effect was PR after 3 cycles.At present,the patient has been very well.
Conclusion: There are a definite reducing of AFP after application of Xiaoaiping, Octretide Acetate, and the tumor definitely reduced after adding the treatment of Jinlong caps.It may be a green therapy for the treatment of the great HCC of octogenarian with Xiaoaiping injection, Jinlong caps and Octretide Acetate.

PP-178
Serological detection of hepatocellular tumor-associated antigen kinectin
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Kinectin was a hepatocellular carcinoma-associated antigen （Stenner-Liewen F，Luo G, Sahin U, et al. Definition of tumor-associated antigens in hepatocellular carcinoma. Cancer Epidemiol Biomarker Prev.2000,9:285-290.） by SEREX (Serological Identification of Recombinant Expression Cloning) techniques.The goal of this research was to detect the positive rate of anti-kinectin antibody in varied diseases serum and assess the diagnosis value of kinectin for HCC. The optimized fragment of kinectin recombinant expressed by our laboratory were coated on the enzyme lable plate.The OD value of anti-kinectin antibody in 150 normal cases and 240 varied diseases cases serum were detected by ELISA techniques.The clinical diagnosis were correlated assay also. The results show anti-kinectin antibody positive cases have been detected in gastric carcinoma (4/11,36%), colon carcinoma (3/15,20%), hepatocellular carcinoma (9/48,19%), breast carcinoma (3/21,14%), leukemia (4/41,10%), lung cancer (2/34,6%), rectal carcinoma (1/18,6%), SLE (1/16,6%), hepatitis B (2/7,29%), hepatocirrhosis (6/29,21%) serum.The sensitivity (18.8%), specificity (92.4%), positive likelihood rate (2.5) of diagnosis for HCC by kinectin have been calculated,and ROC curve for diagnosis of HCC had statistically significant ($P<0.05$). These findings suggested that anti-kinectin antibody can express in some types tumor, SLE, and some hepatopathies serum. Serological detection of anti-kinectin antibody maybe significant to the differential diagnosis for HCC with non-HCC.

PP-179
Targeted anti-inflammatory and angiostatic therapy in pre-treated patients with advanced hepatocellular carcinoma
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Targeting tumor systems biology in patients with advanced and pre-treated hepatocellular carcinoma (HCC) with a combined anti-inflammatory and angiostatic treatment strategy. In a prospective one arm, one stage multi-center phase II trial between 02/2004 and 11/2006 patients with measurable, histologically proven, inoperable, progressive HCC, either treatment-naïve or with local and/or systemic pre-treatment, and Child-Pugh A, received continuous oral pioglitazone 60mg daily, day 1+, etoricoxib 60 mg daily, day 1+, as anti-inflammatory treatment and 1g/m^2 absolute capecitabine bid for 14 days, every 3 weeks as angiostatic therapy (starting day 1+) until tumor progression. Tumor response was assessed every 6 weeks using modified WHO criteria. Primary endpoint was time to progression (TTP). Results: Of 38 patients (pts) treated (male 74%, median age 61 years) CLIP score was 0 (5%), 1 (56%), >1 (39%), 26 pts (68%) were locally (TACE or surgery), 11 (29%) systemically pretreated, 14 pts (26%) received first-line, and 24 pts (74%) second to forth-line therapy. On the basis of independent response assessment 11 (29%) had stable disease for at least 4 months. Disease stabilization occurred in 5 of 13 (38%) locally pre-treated pts, in 4 of 11 pts (36%) systemically (and locally) pretreated, and in 2 of 14 treatment-naïve pts (14%). All pts were eligible for the primary endpoint. Median TTP was 3.4 months (CI 95%, 2.29 to 4.51 months) and median overall survival 6.7 months (CI 95%, 4.08 to 9.32 months). Grade 3/4 drug-related toxicities included edema 13%, hand-foot-syndrome (8%), and anaemia (3%). Conclusions: Although modest efficacy was observed, the

biomodulatory treatment schedule may rescue patients locally and/or systemically pretreated by disease stabilization at a low rate of treatment-associated toxicity.

PP-180

Expression and localization of anchored and anti-hepatocellular carcinoma DNA vaccine containing enhanced green fluorescent protein gene

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Objective: For further functional study on anchored DNA vaccine against hepatocellular carcinoma, recombinant plasmid of pGPC3－EGFP was constructed based on protein engineering theory, moreover fusion protein (GPC3－EGFP) was confirmed expression in eukaryotic cells and localization on cytoplasmic membrane.

Materials and methods: ① pGPC3－EGFP containing three chimeric genes of $hAFP_{542\text{-}550}$ gene, GPI-anchored protein GPC3 gene and enhanced green fluorescent protein gene was constructed; ② GPC3－EGFP was detected on RNA and protein level after pGPC3－EGFP transfected into HepG2 via lipofectamine 2000; ③ EGFP protein was observed under fluorescent microscope using pEGFP-N1 plasmid transfection as a positive control; GPC3－EGFP was also observed by using immunofluorescence technique; Membrane proteins and soluble proteins extracted from transfected 293 cells were detected by western blot using GPC3 monoclonal antibody. **Results:** ① pGPC3－EGFP was constructed successfully by endonuclease digestion and sequencing; ② GPC3－EGFP expressed in HepG2 cells could be detected not only by RT-PCR using specific primers but also by western blot using GFP and GPC3 antibody; ③ Expressed EGFP protein of pGPC3－EGFP was found around HepG2 cells unlike that of pEGFP-N1; Immunofluorescence result also showed red fluorescence around HepG2 cells; Especially western blot results of membrane proteins and soluble proteins indicated GPC3－EGFP could only be detected in membrane protein.

Conclusions: Recombinant plasmid of pGPC3－EGFP could express fusion protein (GPC3－EGFP) in eukaryotic cells, furthermore the fusion proteins could also locate on the cytoplasmic membrane.

PP-181

Study of some biochemical indices of malignancy in ascitic fluide

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Introduction: Ascites derived from Greek word (askos) meaning bag. Disruption of Starling equilibrium (portal hypertension and hypoalbuminaemia) leads to decrease in effective blood volume stimulating renin-angiotensin system, hyper-aldostronism, salt and water retention.Ascites caused by chronic liver disease and malignancy.A.F Cytology in malignancy revealed high incidence of false negative results.

Aim: Estimation of cholesterol, CEA, ALP, LDH, protein, γGT and fibrinogen in A.F to differentiate malignant and non-malignant ascites.

Material: Non-malignant group:-Degree of ascites moderate in 14, tense in 11 cases, diagnosed as schistosomal hepatic fibrosis, post-hepatic cirrhosis, tuberclous peritonitis and nephrotic syndrome.Malignant group:-Degree of ascites ranged from modrate in 17, tense in 8 cases, diagnosed as hepatocellular carcinoma, liver metastasis, gall bladder cancer, cancer colon and pancreatic carcinoma.

Methods: Full clinical examination. Complete urine and stool examination. Examination of A.F: protein, fibrinogen, glucose, chloride, cholesterol, CEA, bilirubin, LDH, ALP, γGT, aminotransferses and test for malignant cells.

Results: There was significant increase in A.F total protein, cholesterol and alkaline phosphatase in malignant than non-malignant group.A.F/serum ratios for protein and cholesterol were significantly higher in malignant than non-malignant group.Diagnostic performances; sensitivity, specificity, predictive value of positive & negative results and efficiency of test for A.F level and A.F/serum ratio for protein & cholesterol were calculated.

A.F protein:54.2,96,92.9,68.6&75.5%.

A.F/serum protein ratio:9.1,100,100,53.5,55.6%.

A.F cholesterol all diagnostic characteristics :100%.

A.F/serum cholesterol ratio:82.6,100,100,88.5,93.3%.

Conclusion: A.F Cholesterol level exceeding 100mg/dl or A.F/serum ratio above 0.7 are highly suggestive of malignancy even in absence of positive cytological findings.Thus A.F cholesterol level A.F/serum cholesterol ratio should be added to monitor malignancy.

PP-182

Clinical therapeutic effects of arsenic trioxide combined with 5-Fu、ADM、Iodized oil in the treatment of middle or advanced hepatocellular carcinoma by interventional way

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Objective: To evaluate the effect and toxicity of arsenic trioxide combined with 5-Fu(Fluorouracil)、ADM(Adriamycin) and Iodized oil in the treatment of middle or advanced hepatocellular carcinoma by transcatheter arterial chemoembolization (TACE) .

Methods: 30 patients with middle or advanced hepatocellular carcinoma were divided into two groups at random. 5-Fu 1.0g, ADM 40mg and Iodized oil 15-30ml were injected into hepatic artery by catheters in 16 patients of A group; based on the medicine used in group A, 20mg of Arsenic trioxide was added in 14 patients of B group. The effect and toxicity were observed.

Results: Af ter 1-3 times of the therapy in group A, objective response rate was 25 % with 4 cases of PR , 10 cases of NC and 2 cases of PD. AFP decreased f rom(61021 ±1203) ng/ mL to (5562 ±102)ng/ mL averagely ($P < 0.05$). In group B, 6 tumors got 25% smaller and 2 tumors got more than 50% smaller in 14 cases after that. AFP decreased from(61125±210) ng/ mL to (2412±125)ng/ mL averagely ($P < 0.05$) .There was significantly different from group A to group B after treatment.The major toxic side effects were pyrexia , nausea and vomiting , pain in hepatic region , mild bone marrow depression and hepatic damage. No irreversible toxic response occurred.

Conclusion: Arsenic trioxide combined with 5-Fu、ADM、Iodized oil in the treatment of middle or advanced hepatocellular carcinoma by TACE is effective and has less adverse effect ,which is a better choice for middle or advanced hepatocellular carcinoma interventional therapy.

PP-183

Synergistic antitumor activity of cetuximab combined erlotinib in human hepatocellular carcinoma cell lines HepG2 and Bel-7402

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Background & Objective: Epidermal growth factor receptor (EGFR) expressed frequently in hepatocellular carcinoma (HCC) and paratumor tissues, associated with oncogenesis and progress of HCC. Cetuximab or Erlotinib alone can inhibit the proliferation of HCC cells. We studied the synergistic antitumor activity of Cetuximab and Erlotinib in HepG2 and Bel-7402 cells and explored the molecular mechanism by Western Blotting analysis

Method: Increasing concentrations of Cetuximab（5～500 mg/ml）and Erlotinib（2.5～250 μmol/L）alone or in combination were added to HepG2 and Bel-7402 cells, the proliferation inhibition effects in different time points were observed, the combination index (CI) of them were calculated. Western blotting analyses were performed to examine the expression of key enzymes in EGFR signaling transduction pathway after different treatments.

Results: After 72 h the maximum inhibition effects of Cetuximab and Erlotinib to HepG2 cell were 43.1±1.9% and 83.4±1.3%, respectively; to Bel-7402 cell were 35.1±2.6% and 73.9±1.2%, respectively. While the maximum inhibition effects of their combination to HepG2 and Bel-7402 cell were 91.1±1.0% and 84.6±1.1%, respectively. The CI values of these two agents in different time points were all less than 1. Western blotting analyses showed that the expression of activated key enzymes (p-EGFR, p-ERK1/2, p-AKT) in EGFR signaling transduction pathway were down-regulated more obviously after combination treatments.

Conclusion: The combination of Cetuximab and Erlotinib has obvious synergistic antitumor activity in HCC cells. The molecular mechanism was further down-regulation of activated key enzymes in EGFR signaling transduction pathway.

PP-184

Role of the KLF6 tumor suppressor in DNA damage response of human hepatocellular carcinoma cells treated with cisplatin

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Background: The molecular mechanism of KLF6 tumor suppression is not well understood. The aim of our study was to explore the potential role of Klf6 in DNA damage response in hepG2 cells.

Methods: After HepG2 cells were treated with cisplatin, subcellular localization of KLF6 protein was examined by immunofluorescence with the anti-KLF6 antibody and expression of KLF6 was detected by RT-PCR and Western Blot. Analyzing alteration of cell cycle was determined by flow cytometry. TUNEL assay were performed to detect apoptosis induced by cisplatin. Host Cell Reactivation assay was used to examine the DNA repair capacity

Results: Nuclear localization of KLF6 protein in HepG2 cell was enhanced following ciplatin treatment. KLF6 expression were upregulated in HepG2 cells when treated with low concentration of cisplatin, and degraded at apoptotic doses. Exposure of HepG2/KLF6 cells to cisplatin 5-10ug/ml for 24 h led to more GI arrest. Exposure to cisplatin to higher concentrations led to slightly increased apoptosis and an slight increase of the number of cells in the sub-GI fraction of the DNA content profile. The ability of the HepG2/KLF6 cells to repair the damaged DNA induced by cisplatin was significantly enhanced.

Conclusion: KLF6 is induced in the setting of DNA damage with cell cycle arrest at G1 phase. When apoptosis induced by cisplatin occur, KLF6 protein is degraded. KLF6 may preferentially activate DNA repair pathways after DNA damage induced by cisplatin**.**

PP-185

The role of neoadjuvant chemotherapy in colorectal liver metastasis

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Background: The aim of this study was to analyse the outcome of patients that received neo-adjuvant chemotherapy prior to resection for colorectal liver metastases (CRLM) and compare them with a matched cohort of patients that underwent resection followed by adjuvant chemotherapy.

Methods: 687 patients have undergone curative resection between January 1993 and January 2006. In this period, 84 patients received neo-adjuvant chemotherapy and 71 of this group went on to resection. A control group was chosen, matched with these patients, made up of patients who underwent resection followed by adjuvant chemotherapy.

Results: There was no difference in clinico-pathological features between the neo-adjuvant and the control group. However patients in the control group had more extended resections and had longer hospital stays compared to those in the neo-adjuvant group, p=0.015. Patients in the control group had an increased incidence of "early" recurrences, p<0.001. Despite this, there was no significant difference in either the cancer-specific or the disease-free survival between the two groups of patients.

Conclusion: Neo-adjuvant chemotherapy has a role in the management of patients with disease that is considered initially unresectable as a "down sizing" technique. In patients with resectable disease, a "test of time" that neo-adjuvant therapy offers is yet to be proven.

PP-186

Cyclooxygenase-2 (Cox2) expression on human benign and malignant hepatocellular tumors

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Background: Recent studies have highlighted the relevance of Cox2 in carcinogenesis. Most researches of Cox2 in human livers used immunostaining and RT-PCR, and suggested cox2 is important in hepatocellular carcinoma (HCC) carcinogenesis. However a few articles show Cox2 is lower in HCC compared to adjacent tissue.

Methods: Real-time RT-PCR and western blot were used to detect mRNA and protein levels of Cox2 on human liver samples, included 8 focal nodular hyperplasia (FNH), 7 telangiectatic hepatic adenoma (T-HCA), 7 hepatocellular adenoma (HCA), HCC (21 in cirrhotic-livers and 9 in non-cirrhotic-livers), and 9 normal histological livers.

Results: mRNA and protein expressions of Cox2 were summarized in figure 1 and 2 respectively. In 4 FNH, Cox2 protein is higher than their adjacent tissues. In 9 of 11 HCC-non-cirrhotic, Cox2 protein expression is lower than their adjacent tissues. Compared to adjacent tissues, Cox-2 protein is higher in 3 HCC-cirrhotic, lower in 1 HCC and equal in the rest 4 HCCs.

Conclusion: Cox2 protein increases in FNH, compared to normal livers and other tumor groups. Compared to normal livers and adjacent tissues, the cox2 mRNA was down-regulated in both HCC groups, and cox2 protein decrease in HCC-non-cirrhotic.

Figure 1. Relative gene expression of Cox2 in normal livers (n = 9), FNH (n=8) and their adjacent liver tissues (n=4), T-HCA (n=7) and their adjacent liver tissues (n=4), HCA (n=7) and one adjacent liver tissue, HCC non-cirrhotic livers (n = 21) and their respective adjacent tissues (n = 11) , HCC in cirrhotic livers (n = 9)and their respective adjacent liver tissues (n = 9). Values are given as mean; error bars represent the standard error of the mean. *P <.05; **P <.01.▩: normal liver, ■: tumor; □: adjacent.

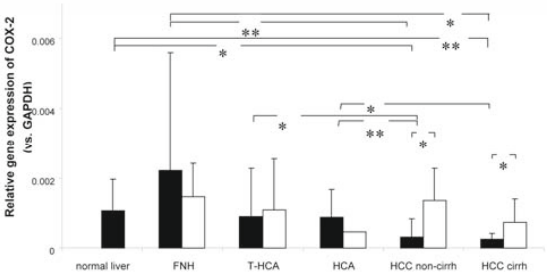
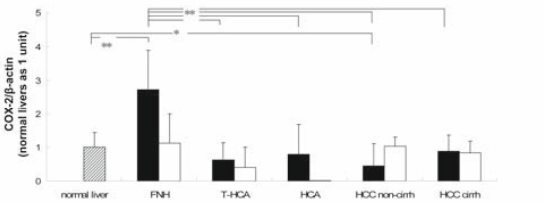


Figure 2. Relative protein level of Cox-2 in 9 cases of normal livers, 8 cases of FNH (with 4 cases adjacent liver tissues), 7 cases of T-HCA (with 4 adjacent liver tissues), 7 cases of HCA (with 1 adjacent tissue), 11 matched HCC arising in non-cirrhotic livers ,and 8 matched HCC arising in cirrhotic livers, as determined by western blot (normal livers as 1 unit). Values are given as mean; error bars represent the standard error of the mean. *P <.05; **P <.01.▩: normal liver, ■: tumor; □: adjacent to tumor.



PP-187

Demographic and clinico-pathological characteristics of egyptian patients with cholangiocarcinoma

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Background: Cholangiocarcinoma (CCA) is a usually fatal malignancy that presents late and has a poor prognosis. Recent epidemiological studies have shown that incidence rates for CCA are increasing worldwide, with substantial regional variation. To date, no studies have examined metabolic profiles in Egyptian patients with CCA.

Aim: The aim of this study was to identify the characteristic demographic and clinico-pathological features of Egyptian patients with cholangiocarcinoma, in order to improve both early diagnosis and ultimate prognosis.

Methods: We collected and reviewed the demographic, clinical, laboratory data imaging and cholangiographic studies and treatment modalities of patients admitted with cholangiocarcinoma in the National Liver Institute (NLI), Menoufiya University, Egypt between October 2004 and November 2005. In order to evaluate the life expectancy of patients, follow up analysis was done from the time of diagnosis until the end of the study. We also compared our data to the data of patients with CCA from the Hammersmith hospital, London.

Results: Fourty-six patients admitted with CCA in the above mentioned period. The median age was 52.2 years. The patients constituted 52.2% distal CCA, 37% hilar CCA and 10.8% intra-hepatic CCA. The prevalence of HCV antibody was 31%. The mean survival time was 298 days.

Conclusion: Egyptian patients with CCA presented at a younger age and had higher bilirubin level than other patients. They presented late, so that curative surgical resection was occasionally feasible

PP-188

Syk(L) and Isoform Syk(S) play different roles in hepatocellular carcinoma patients survival

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The full-length spleen tyrosine kinase (Syk) is known as Syk (L). Short isoform Syk(S) differs from Syk (L) in biological activity in spite of their striking structural similarity. The aim of present study is to investigate the expression status of Syk (L)/(S) in human hepatocellular carcinoma (HCC) and to evaluate this information for its ability to predict disease prognosis. Syk (L) was detected in 8 HCC cell lines and Syk(S) was only found in 2 metastatic HCC cell lines. Syk (L) and Syk(S) were expressed in 84.85% and 31.82% of 132 HCC neoplastic tissues, respectively; 100% and 0% in 30 normal liver tissues without cirrhosis, respectively. The patients with Syk (L) expression in neoplastic tissues had a significant higher overall survival rate after hepatectomy than those without Syk (L). However, the difference in disease-free survival rates was not statistically significant. In contrast, the patients with Syk(S) expression in neoplastic tissues had a statistically significant lower overall and disease-free survival rates than those without Syk(S). Multivariate analyses indicated that factors affecting overall survival were TNM stage, Syk (L) expression status and Syk(S) expression status, and factors affecting disease-free survival were GGT, AFP, TNM stage and Syk(S) expression status. These results indicate that Syk(s) expression and loss of Syk (L) in neoplastic tissues are independent biomarkers of poor prognosis. Syk (L) may be a negative regulator in the initiation and progression of HCC, but Syk(s), a metastasis-related gene in HCC.

PP-189

The value of HS-AFP in the early diagnosis and differential diagnosis of hepatocellular carcinoma

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Objective: To evaluate the value of hepatoma-specific alpha-fetoprotein (HS-AFP) in the early diagnosis and differential diagnosis of hepatocellular carcinoma (HCC).

Methods: HS-AFP and AFP were measured in 105 cases with HCC and 151 cases with benign liver diseases. HS-AFP was separated with a newly developed electrophoresis system by us and determined with Western blot. AFP was determined with chemoluminescence test.

Results: In 105 cases with HCC, the positive rates of HS-AFP and AFP（>200μg/L） were 60% and 50.5% respectively. In the cases with AFP 50μg/L ~400μg/L, the positive rates of HS-AFP were 77.1% in HCC and 13.6% in benign liver diseases. In 151 cases with benign liver diseases, there were 10 cases with positive HS-AFP, and three of them developed HCC during the follow-up. In 11 cases with small HCC (diameter<3cm), the positive rate of HS-AFP were 45.5%, which was significantly higher than that of AFP (18.2%). The sensitivity, specificity, positive predictive value and negative predictive value of HS-AFP for the diagnosis of HCC were 60.0%, 93.4%, 86.3% and 77.0% respectively.

Conclusions: HS-AFP was a better marker than AFP in the diagnosis of HCC. Monitoring HS-AFP in high risk population was useful to the early diagnosis of HCC. HS-AFP played an important role in the differential diagnosis between HCC and benign liver diseases.

PP-190

Dynamic expression and alterations of nuclear factor-κB and tumor necrosis factor-α during malignant transformation of hepatocytes

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Aim: To investigate the kinetic expression and altered mechanism of hepatic NF-κB and TNF-α during the malignant transformation of hepatocytes.

Methods: Rat hepatoma models were induced with 2-fluorenylacetamide (2-FAA) on male SD rats and morphological changes were observed by HE staining. Dynamic alterations of total RNA, hepatocyte nuclear proteins, levels of NF-κB and TNF-αwere quantitatively estimated at different stages of hepatocyte canceration. Rat hepatic NF-κB-mRNA during the malignant transformation of hepatocytes was amplified by RT-PCR. The levels of human NF-κB expression in liver cancer tissues and their matched paracancerous tissues were quantitatively analyzed by ELISA, respectively.

Results: Histological examinations confirmed that hepatocytes in rats fed with 2-FAA showed vacuole-like denaturations at the early stages, then dysplastic nodules appeared at middle stage and finally progressed to tubercles of cancerous nest, which were hepatocellular carcinoma (HCC) with highly differentiation. An increasing tendency of liver NF-κB-mRNA, NF-κB and TNF-α was found from normal liver to precancerous to cancerous tissues during rat the malignant transformation of hepatocytes and HCC progress ($p<0.01$). The expression of circulating or hepatocyte NF-κB was closely correlated with serum or liver TNF-α($p<0.01$). The specific concentration (pg/mg wet liver tissues or pg/μg liver total RNA) of NF-κB expression was significantly higher in HCC than that in their non-tumor tissues ($p<0.01$).

Conclusion: NF-κB signal transduction pathway and inflammatory mediators participated in the malignant transformation of hepatocytes. Reactivation of hepatic NF-κB and abnormality expression of TNF-αcould be useful molecular markers for early diagnosis of HCC

PP-191

Dynamic alteration of apoptosis inhibitory protein NF-κB and Bcl-2 during the course of hepatocyte carcinogenesis

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Objective: To investigate the kinetic expression and altered mechanism of hepatic apoptosis inhibitory protein nuclear factor-Kappa B (NF-κB) and Bcl-2 during the course of hepatocyte carcinogenesis.

Methods: Rat hepatoma models were induced with 2-acetamidoflurene (2-FAA) on male SD rats. Morphological changes were observed by pathological examinations (HE staining). Dynamic alterations of nuclear proteins, NF-κB and Bcl-2 at different stages of canceration were quantitatively analyzed by immuno histochemsitry or ELISA, respectively.

Results: The rat hepatocytes showed vacuole-like denaturation at the early stages, then hyperplastic nodal appearance at middle stage, and finally progression to tubercles of cancerous nest with highly differentiated hepatocyte cancers during development of rat HCC by Histological examinations. An increasing tendency of liver NF-κB and Bcl-2 expression was found from normal liver to precancerous to cancerous tissues during rat hepatoma development and comparison with lower expression in normal controls ($p<0.01$), and closely relationship was found between them ($r=0.73$, $p<0.01$).

Conclusion: The reactivation of NF-κB signal transduction pathway and overpression of hepatic Bcl-2 werer closely associated with occurrence and development of hepatocyte canceration.

PP-192

Regulation of heat shock protein 27 on NF-κB pathway activation is involved in hepatocellular carcinoma cells metastasis behavior

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At present, molecular mechanisms of heat shcok protein (HSP27) in hepatocellular carcinoma (HCC) metastasis remain unclear. In the study, we observed consecutively up-regulated expression of HSP27 in differently metastatic HCC cells (Hep3B, MHCC97L and MHCC97H) and HCC cells migratory and invasive capability was reduced and apoptosis ratio was increased after HSP27 RNA interference assay. We further demonstrated that depletion of HSP27 could suppress NF-κB pathway activation in HCC cell lines, which may be through regulation of HSP27 on activity of IKK complex and IκBα. These findings suggested involvement of HSP27 in HCC cell lines metastasis potentials through regulating NF-κB pathway activation.

PP-193

Osteopontin as a prognostic indicator of hepatocellular carcinoma patients treated with transarterial chemoembolization

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Backgrounds/Aims: This study was intended to investigate the prognostic significance of osteopontin (OPN) in hepatocellular carcinoma (HCC) patients treated with transarterial chemoembolization (TACE).

Patients and Methods: A total of 46 consecutive HCC patients [age (years; median): 59, Male (%): 39/46 (85), etiology

(HBV:HCV:NBNC): 33:4:9] were subjected. Serum OPN levels were measured by ELISA, using the sera obtained just before and 4 weeks after TACE. Serum biochemistry, alpha-fetoprotein (AFP) and dynamic CT scan were checked at a regular interval. The changes of serum OPN levels were evaluated in relation to the tumor response after TACE. Cumulative survival rates were also compared in accordance with the change of serum OPN level following TACE. The decrease of serum OPN level was defined as the decrease more than 10% at 4 weeks after TACE compared to the baseline level. Partial response was defined as the area of lipiodol uptake more than 50% of the tumor at follow-up CT scan determined 4 weeks after TACE.
Results: HCC Patients with the decrease of serum OPN level tended to be associated more frequently with the partial response following TACE. ($p=0.07$) Furthermore, patients with the decrease of serum OPN after TACE showed significantly higher cumulative survival rates compared to those with stationary or increase of serum OPN. ($p<0.05$)
Conclusions: HCC patients whose serum OPN levels decrease after TACE appeared to have better tumor response and consequently longer survival periods. Thus, it is suggested that OPN may be a useful prognostic indicator of HCC patients treated with TACE.

PP-194
Interleukin-10 Gene Single Nucleotide Polymorphisms are Associated with Hepatocellular Carcinoma in Korean Population
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Background: Hepatocellular carcinoma (HCC) is common and main cause of death among Koreans. Genetic factors are also important contributing factors to the development of HCC. Interleukin-10(IL-10) is a powerful Th-2 cell cytokine produced by lymphoid cells that exerts its functions by inhibiting macrophage/monocyte and T-cell lymphocyte replication and secretion of inflammatory cytokines. We examined whether single nucleotide polymorphisms (SNPs) of the IL-10 gene are associated with HCC amomg Koreans. **Method:** We genotyped three SNPs in the IL-10 gene using the pyrosequencing method in 113 subjects with HCC and 265 normal subjects. We evaluated the association of SNPs of IL-10 gene and clinical characteristics such as tumor type, PVT invasion, stage, tumor size, and metastasis.
Result: Three SNPs (-1082, -819 and -592) of IL-10 gene were found to be associated with HCC in two models (codominant, dominant; $p<0.05$ after adjusted age and gender). In haplotype analysis, one haplotypes (ATA) were detected to be significantly different. There was no difference between genotype distribution of IL-10 gene SNPs and tumor characteristics.
Conclusion: These results suggest that the single nucleotide polymorphisms (SNPs) of the IL-10 gene contribute to genetic susceptibility to HCC in Korean population.

PP-195
The effect of TLP on hepatocellular growth
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Objective: To investigate the effect of TLP on hepatocellular growth.
Methods: Real time cell growth detection system was used to determine the growth of Lx2 cell with or without TLP RNAi sequence, and GAPDH RNAi sequence was used as positive control. Forty eight hepatocellular carcinoma tissue and para-carcinoma tissue specimens were collected from the patients with hepatocellular carcinoma during operation. Real-time quantitative PCR was used to detect the TLP, Smad2 and Smad3 mRNA levels, and β-actin and HCCR were used as internal and positive control respectively.
Results: Compared with the negative control, the growth of Lx2 cell with TLP RNAi was significantly inhibited, which was similar with the positive control. The level of TLP mRNA in hepatocellular carcinoma tissue was higher than that of para-carcinoma tissue (12.07±2.62 vs 11.03±1.57, $p<0.05$), which was similar with HCCR expression pattern (15.64±3.55 vs 13.27±3.47, $p<0.01$). While the Smad2 and Smad3 mRNA levels in hepatocellular carcinoma tissue were lower than those in para-carcinoma tissue (7.64±2.61 vs 8.00±2.77; 5.76±1.95 vs 6.41±2.58; $p>0.05$).
Discussion: This result suggests that TLP gene may have the important role in the process of hepatocellular growth and may have certain relationship to the carcinogenesis of hepatocellular carcinoma.

PP-196
Oxidant–antioxidant status in patients with hepato cellular carcinoma (HCC)
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Aim: The exact pro-oxidant and antioxidant status in Hepato Cellular Carcinoma (HCC) patients is still not clear. This work was undertaken to assess oxidative stress and anti oxidant status in patients with HCC.
Materials & Methods: The study was conducted in thirty patients & compared to controls. Erythrocyte GSH was measured by the method of Beutler et al. Ascorbic acid levels were measured by the method of Tietz. Plasma vitamin E levels were measured by the method of Baker H et al. MDA was determined as the measure of thio barbituric acid reactive substances (TBARS). SOD activity in the hemolysate was measured by the method of Misra & Fridovich. Activity of catalase was measured by the method of Beers and Sizer. GP_X activity was measured as described by Paglia and Valentine in erythrocytes and Plasma GST activity was measured as described by Warholm et al. These parameters were measured in thirty patients and compared to controls. Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the student t – test using the stat -view package.
Results: It was observed that there was a significant increase in erythrocyte MDA levels, SOD, GP_X activities and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with hepato cellular carcinoma when compared to controls. No significant difference was observed in the activity of plasma GST in patients with HCC when compared to controls.
Conclusions: The results of our study suggests higher oxygen free radical production, evidenced by increased MDA and decreased GSH, ascorbic acid, vitamin E and Catalase activity, support to the oxidative stress in HCC. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

PP-197
The apoptotic induction of anandamide on MHCC97-H cells
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The aim of this study was to verify whether anandamide (ANA) has a capacity of inducing apoptosis in a hepatocellular carcinoma cell line with high metastatic potential (MHCC97-H), and to investigate the mechanisms.
Methods: The cell apoptosis was tested by FCM; and caspase-3 activity, by caspase-3 Fluorescent Assay Kit; and mRNA expression of bcl-2 and Bax, by RT-PCR; respectively.
Results: With ANA in 100μM，MHCC97-H cells exhibited apoptotic traits from 12.5% to 81.3% when the time increased from 0h to 6h, $P<0.01$. With ANA in 100μM for 6h, caspase-3 activity of MHCC97-H cells was 226±6.4 in Group A (ANA group), 90±4.3 in Group B (additional negative control group) and 149±5.8 in Group C (control group), respectively, suggesting that caspase-3 activity of MHCC97-H cells was markedly increased by ANA and could be inhibited by the inhibitor of caspase-3, Z-VAD-FMK**.** The mRNA expression of bcl-2 could not be detected in MHCC97-H cells whether treated with ANA or not. There was no difference on Bax mRNA expression between ANA group and control group. **Conclusions:** The apoptosis of MHCC97-H cells could be induced by ANA via caspase-3-transducted signal.

PP-198
Dynamic expression characteristics of insulin-like growth factor-II and insulin-like growth factor binding protein-3 during malignant transformation of hepatocytes
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Objective: To investigate the dynamic expression of insulin-like growth factor-II (IGF-II) and IGF binding protein-3 (IGFBP-3) during development of hepatocellular carcinoma (HCC).
Methods: Rat hepatoma models were induced with 2-fluorenylacetamide (2-FAA) on male Sprague- Dawley (SD) rats. Morphological changes of rat livers were observed, the dynamic changes of liver tissues and serum or liver IGF-II and liver IGFBP-3 levels were quantitatively detected. The expression and distribution of liver IGF-II were analyzed by immunohistochemistry.

Results: Rat hepatocytes from granule-like degeneration to a typical hyperplasia to HCC and the progressing increasing of the levels of hepatic IGF-II after induced by 2-FAA. The levels of IGF-II in hepatoma and sera were significantly higher than any of other groups. The positive relationship of IGF-II was found between liver tissues and sera ($P<0.01$). The IGFBP-3 levels in hepatoma rats were significantly lower than those in other groups ($P<0.01$).
Conclusions: Hepatic IGF-II and IGFBP-3 may participate in hepatocyte canceration and the overexpression of IGF-II and inhibition of IGFBP-3 expression could be useful molecular markers for early diagnosis and prognosis of HCC.

PP-199
The Effect of Dou-Chu-hepatoprotective formula in treating the patients with drug-induced hepatic injury
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Object: To study the effect of Chinese Medicine Dou-Chu-hepatoprotective Formula in treating the patients with drug-induced hepatic injury
Methods: 62 out-patients were selected from the clinic of Jiangsu Province Hospital of TCM, including 38 males and 24 females, with the average age at 42.5. All of the patients were diagnosed according to the present revised standard. Treated with oral decoction of Dou-Chu-hepatoprotective Formula, 1 package per day, administered separately in the morning and afternoon. Take 4-week as 1 treatment course, study for 2 treatment courses.
Results: Highly effective in 42 patients, effective in 19 patients, non-effective in 1 patients. Total effective rate is 98.4%.
Conclusion: Chinese Medicine Dou-Chu-hepatoprotective Formula is effective in treating the patients with drug-induced hepatic injury.

PP-200
Analysis of the correlation of 150-kDa oxygen-regulated protein with human hepatocellular carcinoma
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Object: Human hepatocellular carcinoma (HCC) is one of the most mortal tumer. Our previous studies showed that glycoprotein 150-kDa oxygen-regulated protein (150kd ORP) had significant glycosylation alteration between non-tumor human liver cell line Chang liver, non-metastatic human hepatocellular carcinoma (HCC) cell line hep3B and highly metastatic HCC cell line MHCC97H. In the present study, we further validate expression level of this protein and analyse its biological functions in HCC pathogenesis and invasion. Methods: The protein expression levels of 150kd ORP in the three cell lines were determined by western blot and Cell immunochemistry. 150kd ORP gene levels were mensurated with quantitative real-time PCR. We also analysed cell apoptosis and cell invasion status of the transfected HCC cell with 150kd ORP- specific siRNA. Results: The protein and gene expression level of 150kd ORP were significantly upregulated in HCC cell lines comparing with non-tumor human liver cell line; After transfected with specific siRNA of 150kd ORP, HCC cells resulted in an significantly greater apoptosis rate in comparison with control group untransfected cells, however, their invasive potentials were not effected.
Conclusion: 150kd ORP were up-regulated in HCC cell lines, and it might involve in HCC pathogenesis by inhibiting apoptosis of maligant cells. On the other hand, 150kd ORP did not display effects on invasion potential of HCC cells. This study gave a better understanding of the correlation of 150kd ORP and pathogenesis and invasion of HCC, and help to provide a potential therapeutic target molecule for HCC and a candidate marker of HCC detection.

PP-201
New glycome biomarker to detect early stage of liver cancer
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It is well know that glycoproteins are important for maintaining the ordered "social behavior" of differentiated cells in multicellular organisms, alterations in the sugar chains of glycoproteins contribute to the molecular basis of abnormalities such as invasion of tumor cells into the surrounding tissues and their metastasis. Our group took advantage of a recently developed technique, DNA sequencer-based carbohydrate analytical profiling technology (DSA-FACE) to profile total N-glycans of serum proteins. We have been able to analyze the serum samples of over 450 HBV-infected Chinese patients with liver fibrosis or cirrhosis with or without HCC by using this DSA-FACE technology. We found a new glycome biomarker for detection of Hepatocellular carcinoma (HCC) (named as GlycoHCCTest). This new glycome test showed clearly correlation with the presence of tumors and with tumor size (Liu. et al, 2007). When the new GlycoHCCtest was used in combination with AFT test, the accuracy of HCC diagnosis increased dramatically (with 92% of specificity and 77% sensitivity), which succeeds in detecting the liver cancer in more than half of the patients with cirrhosis and cancer of the liver for whom the AFP test was inconclusive. This test would allow frequent and non-invasive analyses to be carried out on cirrhosis patients, which would enable to detect liver cancer in an earlier stage and to closely monitor the development of the disease.
Xue-en Liu, Liesbeth Desmyter, Chun-fang Gao, Wouter Laroy, Sylviane Dewaele, Valerie Vanhooren, Ling Wang, Hui Zhuang, Nico Callewaert, Claude Libert, Roland Contreras and Cuiying Chen (2007) N-glycomics changes in hepatocellular carcinoma patients with liver cirrhosis induced by hepatitis B virus. Hepatology, in press. (Epub 2007 Aug 7)

PP-202
Effect of HBV X gene transfection on apoptosis of QBC939 and its mechanism
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Objective: To investigate the possible correlation of HBV infection and cholangiocarcinogenesis.
Methods: Eukaryotic expression vector containing entire HBx gene coding region, pcDNA3-x was transfected into human cholangiocarcinoma (CC) cell QBC939. Successful transfection of pcDNA3-x was confirmed by RT-PCR and Western Blot. Apoptosis was analyzed by DAPI and flow cytometry. Expression of Bcl-2-related proteins, cytochrome c (Cyt c) and p65 were studied by Western Blot, expression of Fas and c-myc by RT-PCR, transcriptional activity of NF-κB by reporter gene assay and alteration of mitochondria membrane potential ($\Delta\psi m$) using fluorescent probe. Results: The results of DAPI and cytometry flow showed that transfection of pcDNA3-x into QBC939 cells inducing apoptosis. Overexpression of HBx in QBC939 cells induced increase of ratio between Bax and Bcl-XL, loss of $\Delta\psi m$ and release of cytochrome c from mitochondria into cytosol. p65, a important subunit of NF-κB, accumulated in nuclei, and reporter gene assay showed that transcriptional activity of NF-κB increased after overexpression of HBx in QBC939 cells. Fas and c-myc were upregulated at mRNA level after HBx transfection into QBC939 cells.
Conclusion: Our study gives a new view to the research of correlation of HBV infection and cholangiocarcinogenesis.

PP-203
Effect of hepatic blood inflow occlusion without hemihepatic artery control
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Objective: To investigate the effect of hepatic blood inflow occlusion without hemihepatic artery control on the liver cell apoptosis during hepatic ischemia-reperfusion injury in rats.
Methods: 96 Wistar rats were divided into three groups at random. There were Pringle group (group Ⅰ), hemihepatic vascular occlusion group (group Ⅱ) and hepatic blood inflow occlusion without hemihepatic artery control group (group Ⅲ) respectively. Each group 30 minutes hepatic ischemia followed by 1, 2, 6, 24hour reperfusion. Serum ALT, AST, cell apoptosis rate and apoptosis control gene expression were examined. **Results:** After 1, 2, 6, 24hour reperfusion, each time group Ⅰ liver function, liver cell apoptosis rate and Bax、Fas mRNA expression were all higher than group Ⅱ and group Ⅲ（$P<0.01$）. But bcl-2 mRNA expression of group Ⅰ was significantly lower than group Ⅱ and group Ⅲ. The liver function, liver cell apoptosis rate and bcl-2、Bax、Fas mRNA expression of group Ⅱ and group Ⅲ had no significant differences（$P>0.05$）.
Conclusion Hepatic blood inflow occlusion without hemihepatic artery control can reduce the hepatic ischemia-reperfusion injury and the effect is same to hemihepatic vascular occlusion. This method may up-regulate the expression of anti-apoptosis gene bcl-2 mRNA and down-regulate the expression of apoptosis gene Bax、Fas mRNA. So it can reduce the liver cell apoptosis in rat liver ischemia reperfusion injury.

PP-204

CD39 expression by tumor infiltrating lymphocytes suppresses NK-mediated antitumor immunity in the liver

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CD39 (ENTPD1) is the dominant ectonucleotidase of vascular endothelium and T regulatory cells and with CD73 generates adenosine from extracellular nucleotides. Adenosine promotes vascular endothelial cell proliferation and suppresses immune effector responses. Vascular endothelial CD39 expression is required for tumor angiogenesis. Whether CD39 is expressed by tumor infiltrating lymphocytes (TIL) to further impact tumor growth is unknown. Chimeric mice were generated by bone marrow transplants (BMT) from Cd39 null or wild type BL/6 mice. Hepatic metastatic cancer was modeled by portal vein infusion of $1.5x10^5$of luciferase-expressing melanoma B16/F10 cells. Melanoma growth was significantly inhibited with development of tumor necrosis in the livers of all mice that had received Cd39 null BMT. Pharmacological inhibition of CD39 activity, using POM-1, also inhibited tumor growth. Fractions of TIL that expressed CD39 were also foxP3 positive. Importantly, Cd39 null TIL exhibited decreased adenosine production and demonstrated increased cytotoxicity to melanoma cells. Cd39 null CD4+ T cells also inhibited tumor growth after adoptive transfer to Rag1-/- mice where heightened proliferation of endogenous liver NK cells was observed. Tumor inhibition mediated by Cd39 null CD4+ T cells did not occur in the alymphoid mouse strain also deficient in NK cells (null for RAG2/γc).

Conclusions: Antitumor immunity to metastatic melanoma is ultimately NK-mediated but is modulated by the expression of CD39 on TIL cells via local adenosine generation. Pharmacological or targeted inhibition of CD39 may find utility as an adjunct therapy in hepatic or disseminated malignancy.

PP-205

Hilar and peripheralcholangiocarcinomas (CCC) share similar clinico-biological characteristics (European experience)

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Background: Cholangiocellular carcinomas (CCC) can be anatomically divided into, hilar and peripheral CCC.

Objectives: To study the characteristics of both types which have not already been compared in large series

Methods: We report a monocentric series of 168 patients of Hopital Beaujon with histologically proven CCC which included 72 hilar (44%), 92 peripheral (56%) and 4 diffuse hilar and peripheral CCC.

Results: Mean age was 59.5 + 11 years with 91 males (44%) and 77 females (56%). Excessive alcohol and tobacco consumptions were noted in 52% and 42% of patients respectively. When peripheral and hilar CCC were compared, no significant difference was found in terms of age, sex, risk factors, ALT, PAL, AFP, ACE, CA19-9, but patients with hilar tumours shows higher AST (2.7+2.6, vs 1.8+2.5, $p<0.05$), GGT (13.5+14 vs 9.1+11, $p<0.05$), bilirubin levels (113+131 vs 35+69, $p<0.0001$) than peripheral CCC. When available for histological examination, non tumoral liver was histologically normal in 55 patients (45%), or display fibrosis in 48 patients (39%) or cirrhosis in 20 patients (16%) a Distribution was similar for hilar and peripheral CCC. Cumulated survival was poor with 51% of patients alive at 1 year, 22% at 2 years and 6% at 3 years after diagnosis. No difference was observed between hilar and peripheral CCC (Logrank test, $p=0.2$)

Conclusion: No distinctive epidemiologic characteristics were found when hilar and peripheral CCC were compared. Survival in both groups was poor.

PP-206

A new citrus flavonoid compound CF47 retards hepatocellular carcinoma growth by inhibiting cell cycle: results of in vitro and in vivo study

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Objectives: To develop a new class of small molecule antitumor agents of flavonoid class.

Methods: By chemical simulation, a series of flavonoids were designed and synthesized. One of most promising compound, citrus flavonoid 47 (CF47) was systemically studied by in vitro and in vivo essays.

Results: CF47 showed significant tumor inhibiting effects (IC50 0.03 to 0.8 μM) on 6 human tumor cell lines, including mammary adenocarcinoma cells MDA-MB-435s and MCF-7, laryngocarcinoma cell Hep-2, colon carcinoma cell SW480, and highly pulmonarly metastatic hepatocellular carcinoma cell HCCLM7, and human cervical carcinoma HeLa. Moreover, CF47 had greater inhibiting effect on HCCLM7 cells (IC50 0.086 μM) than the most widely used drug 5-flurouracil. CF47 blocked HCCLM7 cycle in the G2/M phase, induced apoptosis and decreased the cell membrane potential, as demonstrated by flow cytometry, DNA ladder essay and electron microscopic studies. Animal toxicity tests confirmed the good safety profile of CF47. When nude mice xenograft model of HCCLM7 were treated with CF47, the tumor growth was reduced by 44.7% at day 35.

Conclusions: CF47 is a newly synthesized cell-cycle targeting agent with confirmed in vitro and in vivo anticancer effect and acceptable tolerability profile.

PP-207

Spiral CT diagnosis of primary sarcomatoid hepatic carcinoma

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Objective: To analyze spiral CT appearances of primary sarcomatoid hepatic carcinoma and discuss its diagnostic value.

Materials and Methods: CT findings of 8 cases with primary sarcomatoid hepatic carcinoma proved pathologically and surgically were analyzed retrospectively. All cases were performed with plain and enhanced spiral CT scan.

Results: Most of the primary sarcomatoid hepatic carcinoma were large mass with diameter between 5cm to 10cm.7 cases had not hepatic cirrhosis basis. 6 cases showed huge cystic-solid lesions with septa(2 cases) or multiple nodulated soft tissues shadows(4 cases),which have different degrees of enhancement. The other 2 cases showed irregular low density lesions with unconspicuous enhancement.3 of 8 cases have cancer embolus of portal vein.

Conclusion: The CT appearances of primary sarcomatoid hepatic carcinoma have doubling characters of hepatic sarcoma and carcinoma. CT signs can show malignant character of lesion, which are helpful to the diagnosis.The confirmed diagnosis depends on pathology and immune histochemistry.

PP-208

Hepatitis B virus X gene upregulates SMYD3 expression in HepG2 cells

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Objective: To explore whether Hepatitis B virus may up-regulate SMYD3 expression in hepatoma cells, through which HBV promotes hepatoma cell proliferation and inhibits hepatoma apoptosis.

Methods: (1) SMYD3 mRNA and protein expression were compared in HBV-positive HepG2.215 cell line and HBV-negative cell line HepG2; (2) Through liposome-oriented transfection, HBX was induced to stably expressed in HepG2 cell. SMYD3 mRNA and protein expressions were tested with real time-PCR and Western Blot respectively. HepG2 cell proliferation and apoptosis were tested with Flow Cytometry.

Results: (1) SMYD3 mRNA and protein expression was significantly higher in HBV positive HepG2.215 than in HBV negative HepG2 cells. (2) After HBX transfection, SMYD3 mRNA and protein were significantly increased in HepG2 cells($P<0.01$), with increased HepG2 cell proliferation and decreased apoptosis.

Conclusion: HBX protein may upregulate SMYD3 expression in Hepatoma. Through SMYD3 pathway, HBX promotes hepatoma cell proliferation, inhibits cell apoptosis.

PP-209

Hepatitis B virus X protein blocks adriamycin-induced apoptosis of hepatocellular carcinoma cells

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Objective: To investigate the effect of HBV X protein on adriamycin-induced apoptosis of HCC cells.

Methods: HBV X gene fragment was inserted into green fluorescent protein (GFP) eukaryotic expression vector pEGFP-C1 to construct recombinant pGFP/HBx. The pEGFP-C1 and pGFP/HBx were introduced into $HepG_2$ cells respectively by Lipofectamine reagent to established $HepG_2$ cell line stable expressing GFP, GFP-HBx fusion protein.The expression of HBV X gene was demonstrated by RT-PCR analysis. $HepG_2$, $HepG_2$/GFP and $HepG_2$/GFP-HBx cells were treated with adriamycin (2.5 μg/ml), and apoptotic cell death was determined by observing morphologic changes, trypan blue exclusion, and flow cytometry analysis.

Results: RT-PCR analysis showed that HBV X gene expressed in $HepG_2$/GFP-HBx cells. Trypan blue exclusion showed adriamycin induced time-dependent cell death in $HepG_2$ and $HepG_2$/GFP cells while no significant cell death was observed in $HepG_2$/GFP-HBx cells. Flow cytometry analysis showed that apoptosis rates in $HepG_2$/GFP-HBx (3.94%) was significant lower than that in $HepG_2$ (59.03%) and $HepG_2$/GFP cells (61.38%) at 36 hours after treatment ($P<0.001$), while no significant difference was observed between $HepG_2$/GFP-HBx (3.94%) and control cells (2.12%, 2.78%, 2.55%) ($P>0.05$).

Conclusions: HBV X protein blocks adriamycin-induced apoptosis of $HepG_2$ cells. It suggests that HBV X is related to the resistance of HCC cells to chemotherapy.

PP-210

A summary of split course field-in-field high target doses radiotherapy for primary huge liver cancer in 20 years.

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Objective: To summarize the new technology of split course field-in-field high target doses radiotherapy for primary huge liver cancer(PLC) .To discuss the value of modern radiotherapy for PLC.

Methods：460 patients with PLC underwent in our hospital between 1987 and 2001 were carried out for retrospective analysis. There were three groups defined by the irradiation doses. Follow-up to Dec, 2006.

Results:（1）260 patients treated with moving split radiation of the whole liver (MSFR) twice, mTD 16~19 Gy. Clinical Benefit Response (CBR) was 82.5% with a median survival duration of 8 months.（2）127 patients treated with MSFR for 3~4 times, mTD 24~35 Gy, CBR was 91.3%, with a median survival duration of 11 months.（3）73 patients treated with moving split radiation of the whole liver and diminution field technology and split course. mTD 50~60Gy obviously reduced more than 40~50%,CBR was100%, 1, 3,5year survival rate 90.2%, 47.9%and 10.9%. 10 patients of them, whose median tumor diameter cytoreduced from 14cm to 7cm, accepted operation after radiotherapy, And the 5-year and10-year survival rate were 40% and 30%, with a longest survival duration of more than 20 years.

Conclusion: The liver is by no means radiotherapy forbidden area. Liver cancer radiotherapy is by no means invalid. split course field-in-field high target doses radiotherapy for PLC, is safety and efficiency, Partial patients have been effect a radical cure. The ascendancy **of** control and killing the micrometastasis which is important for the prevention and treatment of PLC

PP-211

Researches on tumor thrombus of the portal vein in hepatocellular carcinoma

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The molecular mechanism of tumor thrombus in the portal vein (PVTT) is still unclear. We first established a cell line named EHBH08-1 from resected tumor thrombus in portal vein in a patient with histopathologically proved hepatocellular carcinoma (HCC). CD133 cells in this cell line represent one fourth of the tumor cell population and CD133 (+) cells possess a greater colony-forming efficiency, higher proliferative output, and greater ability to form tumor in vivo. With this cell line model and resected tumor thrombi specimen, we also studied the different expression of proteins in primary tumor and tumor thrombus and found 20 proteins expressed differentially between primary tumor and the PVTT. From these proteins, AnnexinV, Prx I , CycB were selected for further analysis to find potential biomarkers of PVTT in hepatocarcinogenesis.

For clinical study, we recommended an uniform tumor thrombus type system (type I～IV) to evaluate or predict prognosis of HCC patients. 406 HCC patients with PVTT were retrospectively analyzed. In the another prospectively control study we conduct a comprehensive treatment by tumor resection + removal of tumor thrombi + TACE + Endostatin for HCC patients with PVTT and have enrolled 20 cases, the period results show good for these HCC patients.

PP-212

Primary hepatic follicular dendritic cell sarcoma with Epstein-Barr virus expression in a case of patient with chronic hepatitis B

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The hepatic follicular dendritic cell sarcoma (FDCS) is an exceedingly rare neoplasm. Only 12 cases have been reported to date. Here, we report a case of hepatic FDCS with Epstein-Barr virus expression and chronic hepatitis B virus (HBV) infection. The clinical, radiological, and pathological findings of this case were described. Histologically, the resected tumor showed an arrangement of fascicle of spindle cells with some Reed-Sternberg like giant cells in a background of predominant lymphocyte infiltration. Immunohistochemical studies showed that the tumor cells were positive for CD21, CD35, CD68, Epstein-Barr virus (EBV) latent membrane protein, but were negative for HBV markers. The diagnosis of the tumor could be confirmed based on the histological features and immunohistochemical staining. Although EBV may be involved in the pathogenesis of hepatic FDCS, in this case, we do not have direct evidence that HBV is associated with hepatic FDCS.

Poster Session – Viral Hepatitis (others) & Miscellaneous

PP-213

The clinical significance of hepatic capsular enhancement in multidirectional computed tomography with Fitz-Hugh-Curtis syndrome

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Background and Aims: Fitz-Hugh-Curtis syndrome (FHCS) is characterized by perihepatitis in patients with pelvic inflammatory disease (PID). Positive immunologic test and hepatic capsular enhancement in arterial phase of abdominal computed tomography are usually required for definitive diagnosis of FHCS. We assessed the clinical significance for intensity of hepatic capsular enhancement in multi-directional CT (MDCT) with Fitz-Hugh-Curtis syndrome.

Methods: Total 82 patients who had typical symptoms and hepatic capsular enhancement in MDCT were enrolled. We excluded the patient with combined cholecystitis (n=2), appendicitis (n=2), pyelonephritis (n=2), pneumonia (n=3). The hepatic capsular enhancement of MDCT findings was divided by 3 patterns (A: partial weak enhancement, B: partial strong or diffuse weak enhancement, C: diffuse strong enhancement). This patterns and perihepatic fluid collection were compared with duration of pain.

Results: Hepatic capsular enhancement in MDCT was classified into 28 of pattern A, 31 of pattern B, 14 of pattern C. Perihepatic fluid collection was visible in 7 of pattern B and 8 of pattern C. The mean duration of pain was significantly different in pattern A (2.9±0.8), pattern B (4.3±1.1), pattern C (5.6±1.2) ($p<0.001$). Duration of pain was well correlated with pattern of hepatic capsular enhancement ($r=0.712$, $p<0.001$) and with perihepatic fluid collection ($r=-0.417$, $p<0.001$).

Conclusions: Hepatic capsular enhancement and perihepatic fluid collection in MDCT is well correlated with duration of pain in patients with Fitz-Hugh-Curtis syndrome. The more intense enhancement of CT may be associated with the increased days of treatment.

PP-214

The effect of CD151 on the HGF/c-Met signal transduction pathway

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Background: It has been reported that the tetraspanin CD151 acts as a promoter of metastasis in several tumors, and plays an important role in c-Met/HGF signal pathway, through forming a functional complex with c-Met, However, the role of CD151 in HGF/c-Met signal pathway in hepatocellular carcinoma (HCC) cells remains unclear.

Methods: To expatiate the mechanism, we investigated the expression of CD151 and c-Met in different metastatic potential of HCC cell lines by RT-PCR and Western blot, and the presence of CD151/c-Met complex in HCCLM3 cells by immunoprecipitation, then modified the CD151 expression by transfection of pcDNA3-CD151cDNA and pGPU6/GFP/Neo-CD151 plasmids into HepG2 and HCCLM3 cells. After CD151 expression in stable transfection HCC cell lines was identified, these cells was treated with HGF (40μg/ml) for one hour, the phosphorylation and non-phosphorylation of Akt, FAK and ERK, which are key molecules in HGF/c-Met signal pathway, were tested by western blot.

Result: The CD151 and c-Met formed a complex in HCCLM3 cells. The phosphorylation of FAK and Akt was higher in high expression of CD151 cells than in low expression ($p<.001$), while the phosphorylation of ERK had no different between the two kinds of HCC cells ($p>.05$).

Conclusion: The CD151 affect the HGF/c-Met signal transduction pathway through the signal molecules of FAK and Akt.

PP-215

Combination therapy with transcatheter arterial chemoembolization and percutaneous radiofrequency ablation compared with percutaneous radiofrequency ablation alone for patients with hepatocellular carcinoma

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Purpose: To assess whether the effectiveness of a combination of transcatheter arterial chemoembolization and percutaneous radiofrequency ablation (TACE-PRFA) was superior to PRFA alone in the treatment of patients with hepatocellular carcinoma (HCC)

Materials and Methods: 240 patients with one to three HCC tumors measuring ≤ 7cm in greatest dimension underwent the combination TACE-PRFA or PRFA alone.

Results: The 1-, 2-, 3-, 5-year overall survival for the groups TACE-PPFA and RFA were 93.4%, 83.4%, 75.4%, 49.7%, and 88.5%, 75.6%, 63.6%, 42.3%, respectively. The survival curve for TACE-PPFA group was better than that for PRFA group significantly (log-rank test, $p=0.045$). On subgroup analyses, the survival curve for TACE-PPFA group was better than that for RFA group for tumors >5.0cm (log-rank test, $p=0.031$), but not for tumors ≤ 5.0cm (log-rank test, $p=0.319$). The survival curve for TACE-PPFA group was better than that for RFA group with 2 or 3 tumors (log-rank test, $p=0.032$), but not for patients with 1 tumor (log-rank test, $p=0.128$). The 1-, 2-, 3-, 5-year PFS for the groups TACE-PPFA and RFA were 89.8%, 76.2%, 63.4%, 41.7%, and 76.3%, 59.5%, 47.0%, 30.2%, respectively. The survival curve for TACE-PPFA group was better than that for PRFA group significantly (log-rank test, $p=0.002$).

Conclusion: In conclusion, TACE-PPFA was better than PRFA in the recurrence control and long-term survival

PP-216

Quantitative measurement of hepatic iron in patients with chronic liver disease

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Introduction: Hepatic Iron overload is observed in many forms of chronic liver disease. Iron can accumulate in the liver in many conditions including congenital, systemic iron-loading conditions (hereditary hemochromatosis), systemic macrophage iron accumulation, in some hepatitidies (hepatitis C, alcoholic liver disease), and uncertain pathogenesis in cirrhosis. This study was conducted to determine the liver iron concentration biochemically.

Materials & Methods: 52 patients with chronic liver diseases referred to gastrointestinal ward were undertaken needle biopsy for measuring iron content. Serum ferritin levels had been measured within 1 month prior to biopsy. SPSS 11.5 was used to analyze data. $P<0.05$ were considered significant.

Results: Liver biopsies from 52 patients (36 male, mean-age 31.7±13-years) were evaluated. The mean hepatic-iron-load (HIL) was 535.8 ±106.1μg/100 mg of dry tissue. HIL had different pattern in secondary hemochromatosis comparing other chronic-liver-diseases. In secondary hemochromatosis, there was a correlation between HIL and the severity of fibrosis, but not the severity of inflammation. In other chronic liver diseases, HIL increased parallel to these indexes:a)Age>40,b) BMI ≥ 25, transferrin saturation and ferritin could increase the HIL; apposite to secondary hemochromatosis, HIL was found to be more in women. There was no relation between HIL and liver enzyme levels.

Conclusions: liver iron content in patients with secondary hemochromatosis (esp. major thalassemia) is very high and these patients need intensive hepatic care.

PP-217

Soft drink consumption limked with fatty liver in the absence of traditional risk factors

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Background: Little is known about dietary habits and their relations to liver disease in NAFLD patients, particularly in the absence of obesity, diabetes or hyperlipidemia.

Objective: Identifying new risk factors permitting early diagnosis of patients with NAFLD.

Methods: 310 patients with NAFLD were assessed in a cross-sectional manner. 31 patients had NAFLD without classic risk factors and were compared to 10 HC. Physical activity and the daily dietary intake of food and soft drink were collected during one week by using validated

food questionnaire by trained dietician. Insulin resistance and lipid peroxidation were assessed by HOMA, and MDA levels respectively.
Results: 80% of patients (25/31) had excessive soft drink beverages (>12 tsp/day of added sugar) for 36 months as compared to healthy controls (p<0.001). 20 % of patients had 1drinks a day, 40% had 2-3 drinks a day, and 40% drank more than 4 drinks a day for most days and for 36 months. The most common soft drinks were Classic Coca-Cola (40 %), Diet Coke (40%), and flavored fruit juices (20% of patients). Ultrasound findings revealed mild fatty liver in 44% cases (n=14), 38% (n=12) moderate, and 18% (n=5) severe. HOMA and MDA levels were significantly higher in patients with NAFLD as compared to healthy controls (HOMA, 3.7 Vs 1.7, P<0.01, and MDA 420±300 Vs 200±100, P<0.001). When controlled for other factors, soft drink beverage was the only independent variables that classified the results correctly in 83 % of cases with 100% SS, 76% SP, PPV 58%, and NPV100%.
Conclusion: Soft drink consumption is common in NAFLD patients with no obvious risk factors. Patients are encouraged to change their longstanding drinking behavior.

PP-218
Clinical utility of the ascitic fluid adenosine deaminase activty in diagnosing tuberculous peritonitis superimposed on liver cirrhosis
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Backgrounds: The diagnosis of tuberculous peritonitis, particularly in patients with chronic liver disease, is usually difficult since liver cirrhosis obscures the clinical features and alters the biochemical characteristics of ascites. The aim of this study was to determine the clinical utility of ascitic fluid adenosine deaminase (ADA) activity in diagnosing tuberculous peritonitis.
Methods: A total of 111 subjects including pure tuberculous peritonitis (n=16), tuberculous peritonitis superimposed on liver cirrhosis (n=8), liver cirrhosis (n=56), liver cirrhosis with spontaneous bacterial peritonitis (n=11), peritoneal carcinomatosis (n=11) and other etiologies (n=9) were analyzed focusing on ADA activity.
Results: The overall sensitivity and specificity of ADA in diagnosing tuberculous peritonitis were 91.7% and 92%, respectively. The accuracy of ADA determination (91.9%) was compared favorably with that of other common ascitic fluid tests. Although absolute ADA activity in 8 patients with tuberculous peritonitis superimposed on liver cirrhosis was lower than that in patients with pure tuberculous peritonitis, all of these patients had higher ADA activities than a cut-off value.
Conclusions: ADA activity determination in ascitic fluid is a useful noninvasive test for the diagnosis of tuberculosis and even in the presence of cirrhosis, the sensitivity of this test is not markedly decreased.

PP-219
Inverse relationship between serum bilirubin and oxysterols, sensitive markers of oxidative stress
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Background: Bilirubin is a potent endogenous antioxidant and clinical studies demonstrate that mildly elevated serum bilirubin levels characteristic for Gilbert syndrome protect from both coronary and carotid atherosclerosis. To analyze whether these effects are related to reactive oxygen species (ROS)-scavenging activities we assessed the relationship between serum bilirubin and 7-keto cholesterol (7keto) and 7-beta hydroxycholesterol (7-bOH). These oxidized derivatives of cholesterol (oxysterols) serve as sensitive *in vivo* markers of oxidative stress.
Methods: The study was performed on 368 healthy subjects of Czech (n=237) and Italian (n=131) origin. The whole population was divided into quartiles according to serum bilirubin levels. Serum oxysterols (determined by GC/MS) among individual bilirubin quartiles were compared (ANOVA on Ranks). Relationships between serum oxysterols and bilirubin were analyzed also by linear regression.
Results: Significant inverse relationship was found between serum bilirubin and both oxysterols (p<0.00001). Subjects with lowest serum bilirubin (1st quartile 2.7-7.1 umol/L) had significantly higher serum 7keto and 7bOH levels as compared to subjects in the highest bilirubin quartile (14.6-48.6 umol/L, p<0.00001 for both oxysterols) as well as 3rd (10.4-14.5 umol/L, p<0.00001 for both oxysterols) and 2nd bilirubin quartile (7.2-10.3 umol/L, p=0.004 and p=0.066 for 7keto and 7bOH, respectively). These inverse relationships were confirmed also in linear regression analyses (p<0.05).
Conclusion: Elevated serum bilirubin levels were inversely related to both serum oxysterols. These data suggest that proposed anti-atherogenic properties of serum bilirubin may indeed be related to its ROS-scavenging activities and that subjects with Gilbert syndrome may be protected from increased oxidative stress.

PP-220
Intrahepatic cholestasis of pregnancy: A 3 year review of 1291 cases
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Objective: Intrahepatic cholestasis of pregnancy (ICP) is reported frequently in China. The true incidence and spectrum are unknown. This study is to summarize the incidence, disease pattern, impact factors and clinical characteristics of ICP.
Methods: This observed data was collected and analyzed retrospectively from the total delivery population during July 1999 to June 2002.
Results: 1291 cases of ICP were found out of 15234 total deliveries. While the overall incidence ICP was 8.4%, there was significant seasonal variation. ICP is more common during months of extreme temperature (hot and cold) than mild months, (9.2%, 9.0% vs 7.5%, P<0.005, P<0.01, respectively). Cholyglycine acid concentrations are increased by 2 to 100 fold. Associated laboratory abnormalities include an increase in fibrinogen (89.5%) and hematocrit (38%) as well as liver enzymes (70%). Adverse pregnant outcomes include preterm delivery (11.7%)，heavy meconium staining (13.2%) and stillbirth(1.4%).
Conclusion: ICP has a high incidence in Southeast China characterized by laboratory abnormalities, adverse pregnant outcomes, and seasonal variation.

PP-221
Diclofenac versus allopurinol for the prevention of post-ERCP pancreatities: A Prospective randomized controlled trial
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Background: Pancreatities is the most common complication following ERCP, which can on occasions be severe and life threatening.
Aim: To compare the efficacy of diclofenac, allopurinol 300 mg and allopurinol 600 mg for the prevention of post-ERCP acute pancreatitis
Patients and Methods: 130 patients were scheduled for ERCP. Patients were randomized to receive a single dose of either: 100 mg diclofenac suppository immediately after ERCP (40 patients, 25 males, 51.8±14.6 years), 300 mg oral allopurinol one hour before ERCP (30 patients, 16 males, 53.3±11.5 years), 600 mg oral allopurinol one hour before ERCP (40 patients, 24 males, 47.6±14.3 years) or no prophylaxis (20 patients, 14 males, 46.9± 14.4 years). Serum amylase and lipase were measured immediately before, and 4 and 24 hours after ERCP.
Results: None of patients on diclofenac or large dose allopurinol developed post-ERCP pancreatitis, versus one patient on allopurinol 300 mg and two in the control group. Serum amylase and lipase increased significantly after ERCP in the diclofenac, allopurinol 300 mg and the control groups (p<0.05). In the allopurinol 600 mg group, serum amylase increased significantly at 4 hours after ERCP while serum amylase at 24 hours and serum lipase at 4 and 24 hours did not.
Conclusions: high dose allopurinol (600mg) prevented the increase in serum amylase at 24 hours and serum lipase at 4, and 24 hours after therapeutic ERCP. Both diclofenac and allopurinol 600 mg were associated with low incidence of post–ERCP pancreatitis

PP-222
A retrospective analysis of the safety of outpatient percutaneous liver biopsies with Von Willebrand Disease
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Introduction: Liver biopsy remains the gold standard for diagnosis of chronic liver diseases. Outpatient percutaneous biopsy is generally safe with a mortality rate of 0.17% and hospitalization rate for bleeding of 3%. Von Willebrand (VW) syndrome is the most common inherited hematological disorder with a prevalence of 1-3% globally. Whether

VW syndrome increases the risk of bleeding in invasive procedures is not known.
Methods: All patients (n=120) who underwent outpatient percutaneous liver biopsies from 1997 to 2007 were analyzed. Demographics, PT/INR, platelet count, VW factors (VW Factors, VWAntigen), Ristocetin cofactors and Factor VIII in serum were collected. Patients had not received ASA, NSAIDs or blood thinners for at least 5 days prior to biopsy. Exclusions included prior coagulation diathesis, familial bleeding history, arteriovenous malformations and vasculitis.
Results: 66 patients (55%) had hepatitis C, 24 (20%) hepatitis B, 10 (8.3%) alcoholic hepatitis, 20 (16%) other diagnoses. 30 (25%) had minor local bleeding that resolved with pressure. 53 (48%) had biopsy site ecchymosis after 24 hrs. 12 (10%) had VW factor deficiency, 5(41%), 7(56%), and 0 had Type I, II and III respectively. No VW patients had bleeding requiring transfusion, hospitalization or surgery but 9 (75%) had minor local bleeding and all had ecchymoses, which resolved spontaneously in a week.
Conclusion: Patients with VW factor deficiency can undergo percutaneous liver biopsy without major bleeding. Minor bleeding may occur at a slightly higher rate. VW syndrome is not a contraindication to percutaneous liver biopsy.

PP-223
Is reagent strip testing of ascitic fluid useful for the rapid diagnosis of spontaneous bacterial peritonitis?
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Background/Aims: A recent large multicenter study refuted the diagnostic efficacy of Multistix 8SG®. Nevertheless, the usefulness of urine strip tests in the diagnosis of SBP remains controversial.
Methods: Between September 2006 and August 2007, 213 consecutive cirrhotic patients admitted with ascites, who underwent therapeutic or diagnostic paracenteses of ascites, were included in this prospective study. A sample of the ascitic fluid was analyzed using two different reagent strips (UriSCAN® and Multistix 10SG®), the conventional PML count, Gram stain and cultures. Different cut-off points indicating positive result in the reagent strip testing were examined.
Results: In all, 686 paracenteses from 213 patients were analyzed. We diagnosed 45 cases of SBP in 28 patients. The concordance between the investigators and nurses was excellent (K=0.840; P < 0.001).
Conclusion: This study suggests that reagent strip testing of ascitic fluid is useful as a simple, adequate bedside diagnostic method for screening SBP.

Urine strip	Lowest grade considered "Positive"	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Multistix 10SG®	**Trace**	100	99.2	90.0	100
	1+	91.1	99.7	95.3	99.4
	2+	62.2	99.8	96.6	97.4
	3+	37.8	100	100	95.8
UriSCAN®	**1+**	100	99.7	95.7	100
	2+	84.4	100	100	98.9
	3+	57.8	100	100	97.1

PP-224
Experimental study of protective effect of PGE1 in the hepatic ischemia reperfusion injury in fat liver rats
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Objective: To investigate the effect of PGE1 on the ICAM-1 expression in ischemic reperfusion injury of fat rat liver.
Methods: 42 Male Wistar rats established fat liver model were randomly divided into 2 groups: the PGE1 group and the control group. Polyethylene catheter was inserted into the branch of superior mesenteric vein, in PGE1group which gave 5ug/mlPGE1 0.1ml/kg/min, 15min before the ischemic reperfusion injury, in control group rats was replaced by normal sodium. The hepatic hilum was blocked for 15 min use Pringle's method. The serum levels of ALT, AST and LDH were measured by whole automatic biochemistry analyzer. The expression of ICAM-1 in liver tissue was measured by immunohistochemistry method.
Results: The ALT and AST level in PGE1 group are lower than those in control group (P<0.05), at 1 hour after reperfusion, the LDH level in PGE1 group is lower than that in control group (P<0.05), but there was no significance at 6 and 24 hours after reperfusion (P>0.05). The ICAM-1 expression in liver tissue of control group was significantly increased than that in PGE1 group (P<0.05) after reperfusion for 1, 6 and 24 hours.
Conclusions: PGE1 might confer the protection to ischemic reperfusion injury of fat rat liver through reducing the expression of ICAM-1.

PP-225
Role of helicobacter pylori infection in expression of inducible nitic oxide synthase in patients with liver cirrhosis
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Background and Aim: Significant nitric oxide NOS enzyme activity, especially the inducible form iNOS has been reported in patients with portal hypertensive gastropathy PHG. On the other hand, increased iNOS expression has been reported in patients with H .Pylori –positive gastritis .So the effect of H.Pylori on iNOS expression in the gastric mucosa of patients with liver cirrhosis LC and the relationship between PHG and iNOS in H .Pylori – negative LC patients is examined
Methods: Sixty patients with LC and 21 healthy matched controls are studied H Pylori is diagnosed by histology and /or culture. PHG is diagnosed endoscopically and is classified according to the Baveno111 classification into mild and severe forms. In all patients biopsy is obtained from the gastric antrum and body for histological and immuno-histological examination and for H .Pylori culture
Results: PHG is significantly associated with the presence of esophageal varecies, gastric varecies and history of sclerotherapy. However, the frequency and severity of PHG are independent of the patient's age, sex, etiology or severity of LC, H Pylori infection, mucosal capillary dilation, nor degree of histological gastritis. H .Pylori induces significant iNOS expression in the gastric mucosa of both LC patients and controls. On the other hand, significant iNOS expression is observed in severe than mild PHG irrespective of H .Pylori status.
Conclusion: Both H Pylori and PHG independently induce significant iNOS expression in the gastric mucosa of LC patients, however, they have no additive or synergistic effect on this expression.

PP-226
Meld-Na and MELD score for predicting the development of spontaneous bacterial peritonitis in liver cirrhosis
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Background/Aims: To determine the relationship between Child-Pugh score (CPS), MELD score, MELD-Na (MELD with incorporation of serum Na) and the development of spontaneous bacterial peritonitis (SBP) in patients with liver cirrhosis.
Methods: Clinical data of 321 cirrhotic patients with ascites who underwent diagnostic paracentesis upon hospital admission (March 2002-July 2007) were retrospectively reviewed. SBP was defined as a paracentesis yielding ≥ 250 neutrophils/mL ascites fluid after excluding other causes. The prediction for the development of SBP was analyzed using the area under the receiver's operating characteristics curve (AUC) according to CPS, MELD score and MELD-Na.
Results: One hundred twenty four (38.6%) of 321 hospitalized patients with cirrhotic ascites were found to have SBP. The causes of cirrhosis were viral hepatitis (57.3%), alcohol (39.3%) and others (3.4%). Overall CPS, MELD score and MELD-Na correlated significantly with the presence of SBP (r = 0.249, p<0.001 vs. r = 0.372, p<0.001 vs. r = 0.408, p<0.001, respectively). The mean CPS, MELD and MELD-Na in patients with and without SBP were 10.6 ±1.46 and 9.8 ± 1.72, 23.0 ±7.58 and 17.4 ± 6.16, 31.1 ± 13.36 20.8 ± 9.56, respectively (all, p< 0.001). The AUC of CPS, MELD score and MELD-Na for predicting SBP were 0.643, 0.718, and 0.739, respectively (p<0.001). Odds ratio for the development of SBP were 4.00 (95% CI, 2.34-6.82, p<0.001) and 10.80 (95% CI, 5.12-22.78, p<0.001) in patients with MELD-Na of 16-26 and ≥ 27, as compared to those with MELD-Na ≤ 15, respectively.
Conclusion: The results show that MELD-Na is slightly better than MELD score and CPS for predicting the development of SBP. The increasing MELD-Na is significantly related to the increasing risk of SBP development in patients with liver cirrhosis.

PP-227
Modulation of Nrf2-Dependent heme oxygenase-1 contributes parthenolide-induced cholangiocarcinoma cell apoptosis

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Background/Aims: Cholangiocarcinomas (CC) are intrahepatic bile duct carcinomas with poor prognosis. The aims of this study were to clarify the molecular mechanisms whereby parthenolide efficiently induces apoptosis in CC cells and to explore the molecular pathways to enhance this susceptibility.
Methods: The effects of parthenolide on apoptogenicity and altered expression of HO-1 were examined in all four CC cell lines. Its apoptogenic mechanisms were also examined by HO-1 or Nrf2 inhibition.
Results: The low dose (< 40 μM) of parthenolide led to Nrf2-dependent HO-1 induction in CC cells and its apoptogenic effect was relatively low. However, a high dose (> 40 microM) of parthenolide increases its apoptogenic effect, accompanying a decrease of HO-1 induction. Furthermore, this parthenolide-induced apoptosis was synergistically enhanced by PKC-alpha inhibition, but not by HO-1 inhibition. PKC-alpha inhibitor led to inhibition of Nrf2-dependent HO-1 expression, but HO-1 inhibitor ZnPP caused Bach1-dependent HO-1 overexpression.
Conclusions: Partehnolide-induced apoptosis is strongly associated with the inhibition of Nrf2-dependent HO-1 induction, which is enhanced by the PKC-alpha inhibition in CC cells. Thus, the modulation of Nrf2-dependent HO-1 induction may be a novel anticancer therapeutic approach in CC.

PP-228
Laparoscopic liver resection with prior vascular control : a comparative study
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Background: The magnification of laparoscopy allows a very precise dissection of the portal pedicles. The aim of this study was to evaluate the effect of prior vascular control (PVC) on parenchymal dissection in 136 consecutive patients undergoing laparoscopic liver resections.
Methods: Between 1999 and 2006 we performed 136 laparoscopic liver resections. Portal vessels and the concerned hepatic vein were controlled and divided extraparenchymally before liver division, in all formal anatomic resections. Operative duration, blood loss, conversion rates and postoperative complications were compared with patients undergoing laparoscopic non anatomic resections without PVC.
Results: We studied 64 laparoscopic liver resections with PVC and 72 without PVC. There were 39 major resections (>3 segments) in the PVC group and only 7 in the group without PVC. There was no difference in operative time between the two groups (244 vs. 218 minutes for PVC and non-PVC, respectively; p=0.81). Eight patients underwent conversion, only one in the PVC group and 7 in the group without PVC (p=0.06). Blood loss and transfusion rate were significantly lower in the group with PVC (201 vs. 541 ml, p<0.001; transfusion: 3 vs. 17%, p=0.03). Morbidity, specific and general, was not different between the two groups (PVC, 4%; non-PVC, 12.5%; p=0.13). Duration of hospital stay was similar in both groups.
Conclusion: In our experience, blood loss and transfusion rates were significantly lower when a PVC was used despite the higher percentage of major resections in this group. In laparoscopic surgery, liver resection appears to be safer when PVC is performed.

PP-229
Clinical observation of sorafenib monotherapy for Chinese advanced hepatocellular carcinoma
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Objective: To observate the efficacy and safety of sorafenib monotherapy for Chinese advanced hepatocellular carcinoma (HCC) patients.
Methods: Patients with advanced HCC whose Child-Pugh status A or B were included in this study. Patients were administered 400 mg of sorafenib bid on a continuous schedule · Data for analysis of efficacy and safety were collected every four to six weeks.
Results: 26 Chinese advanced HCC patients were included. Macroscopic vascular invasion was present in 11 (42.3%) subjects and extrahepatic spread was present in 13 (50.0%) of patients. The median treating span was 79 days (range: 20-804 days) ，and the median overall survival span was 190 days (range: 58-804 days). The median response duration was 119 days (range: 37-506 days) in 12 patients with response (PR+MR+SD). The most frequent categories of treatment-emergent adverse evens were dermal (19 cases, 73.1％), constitutional (14 cases, 53.8％), and gastrointestinal (12 cases, 46.2 ％). Most adverse events were mild, easily to manage and reversible.
Conclusion: Sorafenib could be well tolerated and beneficial for Chinese advanced HCC patients even who failured from prior treatments or present with extrahepatic metastases.

PP-230
Swine hepatitis E virus in rural southern china: genetic characterization and experimental infection in rhesus monkeys (Macaca mulatta)
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In rural areas of southern China, where hepatitis E is endemic, residents generally rear pigs in pigsties near their houses, and hepatitis E virus (HEV) infections are suspected of being acquired primarily through contact with swine. To assess this, a genetic analysis of swine HEV isolates from rural southern China was performed and the potential risk of cross-species infection of swine HEV was determined. Of 120 swine fecal samples collected from pigsties, 29 were positive for HEV RNA. The nucleotide sequences of these swine HEV strains shared 85%-99% identities with the local human genotype 4 isolates and belonged to two subgroups of genotype 4. Importantly, swine HEV strains representing both subgroups induced hepatitis after inoculation into rhesus monkeys, evidenced by elevated serum alanine transaminase (ALT) level, viremia, fecal viral shedding, anti-HEV seroconversion, and liver histopathological changes. In conclusion, swine may be the principal reservoir for human HEV infection in rural southern China.

PP-231
Paper and pencil or computer assessment of minimal encephalopathy
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A lack of standardized tests was cited by hepatologists for not testing for minimal hepatic encephalopathy. We therefore compared well established paper and pencil tests with a computer based battery (Cognitive Drug Research; CDR) which allows specific cognitive functions to be assessed.
Methods: 89 cirrhotic patients were studied. Composite scores were calculated from the CDR subtests to reflect five cognitive domains and results validated by comparison with those from six standard paper and pencil tests. Level of impairment was defined using the sum of a range of standard deviations by which each CDR domain (CDRS) and each paper and pencil test (PHES) differed from normal age matched controls. CDRS and PHES were repeated in 21 patients after liver transplant and CDRS in 24 patients 3 hours after a 108 g amino acid challenge.
Results: There was a high correlation between the two measures (r=0.748; p = 0.001). Using multiple regression, MELD (p=0.011) correlated with PHES paper and pencil results. In contrast, the CDR domains Continuity of Attention and Quality of Episodic Memory were significantly related to venous blood ammonia levels ($Radj^2$=0.200;F(6,76)=4.41;p=0.001). There were marked deteriorations in the CDR composite scores representing accuracy of Working (p=0.005) and Episodic Memory (p=0.001) after amino acid challenge when blood ammonia increased from 63±36 to 126±62umol/l(p=0.001). Both PHES and CDRS returned to the control range after liver transplantation (PHES:pre-6;post0;p<0.001; CDRS:pre-6;post-2;p=0.003 respectively).
Conclusion: CDRS is valuable for the recognition of minimal hepatic encephalopathy.

PP-232
Laparoscopic versus open right hepatectomy: a comparative study
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Background: Right hepatectomy remains a major procedure, with many steps difficult to perform by laparoscopy. We developed an entirely laparoscopic technique for achieving formal right hepatectomy, following exactly the same steps as the open surgery procedure used in our department for more than a decade.

Methods: Twenty-two successive patients underwent laparoscopic right hepatectomy (LRH) from 2002 to 2007. They were matched with 52 patients retrospectively selected from our open right hepatectomy (ORH) database, for the following criteria: sex, age, ASA score, BMI, liver disease and tumor size. Surgical and postoperative outcomes were compared for the two procedures.

Results: LRH was successfully performed in 20 patients and conversion to laparotomy was required in two cases (9%). Results were analyzed on an intention to-treat basis. Operating time was similar in the two groups (LRH, 360 min; ORH, 329 min; p=ns). Blood loss was significantly less in laparoscopic resections (LRH, 519 ml; ORH, 776 ml; p=0.01), despite the absence of clamping during laparoscopy. However, there was no significant difference in transfusion rates (LRH, 13%; ORH, 17%; p=ns). Specific morbidity rates were similar (LRH, 4.5%; ORH, 11.5%; p=ns), but general morbidity rates were significantly lower after laparoscopy (LRH, 9%; ORH, 32%; p=0.04). Postoperative hospital stay was significantly shorter after laparoscopy (LRH, 8.2 days; ORH, 12.6 days; p=0.007).

Conclusions: Laparoscopy improved surgical and postoperative outcomes for right hepatectomy. This is the first comparative study to demonstrate a clear advantage of laparoscopy for a major liver resection.

PP-233

Aetiological analysis of hepatic diseases causing oesophageal varices in a cohort of adult Sri Lankans and their implications

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Objectives: To assess the aetiology of hepatic diseases in a cohort of adult Sri Lankans, who were endoscopically proven to have oesophageal varices, admitted to a medical unit in a tertiary referral center.

Design and Setting: 2015 upper gastrointestinal endoscopies performed at pricipal author's unit at Sri Jayewardenepura General Hospital, Sri Lanka, for various reasons from 1.3.2002 to 31.12.2004 were retrspectively reviewed. Those who had oesophageal varices were separated and analysed with respect to aetiology.

Results: 376 patients had oesophageal varices and 244 were non-alcoholics (65%) with a sex distribution of male:female 2.6:1 and a mean age group of 55.7 SD± 13.6 years respectively. The same for alcoholic group were 31:1 and 52 SD± 9.8 years. All had evidence of cirrhosis evident by either histology or ultrasonic appearance together with supportive liver function tests. Aetiological analysis of non-alcoholic group showed prevalence of haemochrmatosis in 4, autoimmune chronic active hepatitis in 3, portal vein thrombosis in 3, Wilson's disease in 2, Hepatitis B in 2, Hepatitis C in 1. There were no clinical evidence of other rare metabolic disorders, in non-alcoholic group. 43% of the varices were detected on active surveillance.

Conclusions: Aetiology of non-alcoholic cirrhosis of adult Sri Lankans remains largely unknown, which revealed to be the dominant cause of cirrhosis of oesophageal varices, notably hepatitis B and C infections were rare, thus causing difficulties in formulating preventive strategies

PP-234

Demographics, aetiology, clinical and histological profile of asymptomatic elevation of hepatic transaminases due to NAFLD in a cohort of non-alcoholic adult Sri Lankans

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Objectives: To evaluate the various aspects of asymptomatic elevation of hepatic transaminases due to NAFLD in adult Sri Lankans.

Design and setting: Case notes of 122 consented liver biopsies performed in principal author's unit at Sri Jayawardenepura General Hospital Kotte, Sri Lanka, in patients investigated for above abnormality from 17/04/2002 to 31/7/2007 were retrospectively analyzed, using diagnostic criteria of NAFLD.

Results: Study population had an age group of 17-61 yrs with a mean of 37.5±SD12.9yrs. Male: female sex ratio was 92:30 ≈3:1. Histology revealed steatosis, NASH, NASH with cirrhosis in 80.7%, 15.2%, and 4.1% of instances respectively. Associated diabetes mellitus, hyperlipidaemia, and hypertension in various subgroups were as follows; steatosis: 61.5%, 72.2%, 68.4%; steatohepatitis: 30.7%, 22.2%, 26.3%, NASH with cirrhosis: 8.8%, 5.6%, 5.5% respectively. Mean ages and BMIs of presentation for males and females were 35.7±SD 13.3 yrs, 23.0±SD2.4, 46.0±SD7.0 yrs, 22.8±SD1.3 respectively. Mean ages and BMIs for the above subgroups were as follows. Steatosis 36.3±SD13.5yrs, 22.7±SD2.4, NASH 42.7±SD5.1yrs, 25.6±SD2.2, NASH with cirrhosis 47.3±SD7.5, 24.8±SD 2.2 respectively. Social status had no influence. Lack of optimal exercise was noted in all.

Conclusions: Diabetes, dyslipidaemia and hypertension were most dominantly associated with steatosis implying a cluster of metabolic syndrome. Higher BMI values were seen with the presence of NASH and NASH with cirrhosis. Cirrhosis was detected relatively early in the cohort, which was alarming. Mean age of presentation for females was late and mean BMI for both sexes showed no significant difference for the spectrum of NAFLD

PP-235

Major bile duct surgery: Is it a predisposing factor for vanishing bile duct syndrome? Two case reports

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Background: Acquired vanishing bile duct syndrome (VBS) is a rare hepatic disorder associated with a variety of conditions, which has a grave prognosis. We report two cases, of adult Sri Lankans who developed the above condition following surgery involving major bile ducts.

Case Report 1: 58 year old male was investigated for vague ill health and a diarrhoeal illness in September 2005. He was found to have mild diabetes and extensive investigations were unfruitful except for a non specific colitis of colonic biopsies. Six months later he presented with icterus and investigations revealed a left hepatic duct cholangiocarcinoma, and underwent partial hepatectomy. Hepatic histology showed tumor free margins, and he clinically improved dramatically. Later he re-presented with obstructive jaundice, without evidence of recurrence of tumor and compensatory hypertrophy of the right hepatic lobe. Liver histology showed VBS. He succumbed to his illness months later.

Case Report 2: 38 old mechanic was referred for ERCP evaluation of obstructive jaundice in august 2007. He gave a past history of exploratory laparotomy for stab injury and subsequent choledocho-duodenostomy for a benign terminal common bile duct stricture. Investigations revealed obstructive jaundice, non obstructive ERCP findings with a pancreatico-gastric fistula. Liver histology showed VBS. In both all other relevant investigations were normal.

Conclusions: Surgery involving major bile ducts seems to be a predisposing factor for development of VBS. The postulated mechanism is exposure of bile duct antigens, during surgery, triggering off a destructive autoimmune reaction targeting the biliary tract.

PP-236

Liver dysfunction in acute pancreatitis: role of antioxidant therapy and evacuation of pancreatitis-associated fluid collections

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Introduction: The liver damage is an important prognostic sign of severe pancreatitis. The hepatic dysfunction that can occur during severe acute pancreatitis presents with elevated serum hepatic enzymes, hepatocellular acidosis, and changes in the microvascular environment of the hepatic parenchyma.

Materials and methods: Plasma levels of IL-1β, TNF-α, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase (LDH), and glutathione were measured in 55 patients (33 – severe and 22 – mild pancreatitis).

Results: Liver dysfunction was noted only in patients with severe pancreatitis that accompanied by increased levels of LDH and ALAT. The clear correlation between proinflammatory mediators' concentration and enzymes (LDH, ALAT) activity was observed. The glutathione level decreased in all patients with acute pancreatitis, but the lowest levels were noted in patients with severe pancreatitis. The reverse correlation between ALAT and glutathione was noted. The liver function significantly improves after removing of pancreatic fluid collections and infusions of ademetionine (Geptral) – 800 mg/daily. Evacuation of pancreatitis-associated fluid collections also improves

function of kidneys and lungs, reduces risk of infected necrosis (χ^2=11,50; p=0,0007).
Conclusion: These results pointed on participation of pancreatitis-associated ascitic fluid in the development of liver dysfunction. Microcirculatory liver dysfunction resulted in the additional liberation of proinflammatory mediators, neutrophils accumulation in sinusoids, oxidative stress that leads to the hepatocytes damage and parenchimatious insufficiency. Thus the evacuation of fluid collections and applying of glutathione precursors are necessary.

PP-237
Rifaximin versus non-absorbable disaccharides in the management of hepatic encephalopathy: a meta-analysis
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Objective: To assess the efficacy and safety of rifaximin versus current first-line drugs—non-absorbable disaccharides in patients with hepatic encephalopathy (HE).
Methods: We employed the method recommended by the Cochrane Collaboration to perform a meta-analysis of randomized controlled trials (RCTs) irrespective of language, publication status, or blinding of rifaximin versus non-absorbable disaccharides in the management of HE.
Results: 7 RCTs met our selective criteria. When compared the numbers of patients with clinical efficacy of HE, relative risk (RR) 1.08 [(95 percent CI 0.85 to 1.38); p = 0.53] with no significant difference between rifaximin and non-absorbable disaccharides. When considering adverse events, we found there was no significantly different between rifaximin and non-absorbable on abdominal pain (RR = 0.41, 95 percent CI 0.15 to 1.13) but significantly different on diarrhea (RR = 0.16, 95 percent CI 0.05 to 0.52).
Conclusion: the present study shows rifaximin is not superior when compared with non-absorbable disaccharides with acute or chronic HE for long or short treat-term. But it may be with higher tolerability. Further studies on larger populations of patients are necessary to give more sufficient evidence for the usefulness of rifaximin.

PP-238
Pre-existing liver cirrhosis reduced the toxic effect of diethylene glycol in a rat model
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Objectives: Diethylene glycol (DEG) poisoning is life-threatening. It is worthy to clarify the potential influences of the pre-existing liver disease on it since liver is the main location of DEG metabolism.
Methods: Forty model rats with carbon tetrachloride-plus-alcohol-induced liver cirrhosis and 20 control rats were intraperitoneally given a single dose of 4 ml/kg body weight of DEG. Blood, 24-hour urine or tissue samples were collected for examinations of liver and renal function profiles, DEG concentration, pathological changes or hepatic of ADH and ALDH activities.
Results: Compared to control rats, the model rats had significantly lower mortality on the eighth day, higher blood CO_2-combining power and lower blood urine nitrogen, serum creatinine and alanine aminotransferase levels on the second day. Pathological examinations showed hepatocyte degeneration and renal tubule necrosis in model rats were also comparatively less serious. The excreting DEG of the model rats on the first day was significantly higher than that of control rats (46.65±8.79 mg vs 18.88±6.18 mg, P<0.01). While, the DEG concentrations in blood and tissue samples were relatively lower thereafter. The hepatic ADH activities of model rats were significantly lower than those of control rats. By analyzing using Spearman's rank correlation, hepatic ADH was positively correlated (r_s=0.582, P<0.01) to the serum creatinine level.
Conclusion: The toxic effects of DEG were all substantially reduced by pre-existing liver cirrhosis in rat model. Its possible mechanism was due to the impaired hepatic ADH activity. These findings might be helpful in toxicological researches and clinical practice.

PP-239
Serum leptin does not correlate with hepatic fibrosis in patients with nonalcoholic fatty liver disease (NAFLD) and chronic hepatitis C (CHC)
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Background: Human data on the role of adipokine leptin in hepatic fibrosis is controversial. Aim of this study was to measure serum leptin levels in patients with NAFLD and CHC and correlate its levels with hepatic fibrosis.
Methods: Serum leptin levels were assessed by ELISA (DRG Diagnostics, Germany) in 40 patients with NAFLD (Mean age 39.8±10.8 years, Men 29), 23 patients with CHC (Mean age 37.7±6.7, Men 22) and 15 healthy controls (normal BMI, LFT and ultrasound abdomen), (Mean age 32.2±9.2 years, Men 13). Histological analysis was done as per Matteoni et al (Gastroenterology 1999), Brunt et al (Am J Gastroenterol 1999) and Ishak et al (J Hepatol 1995) in patients with NAFLD and CHC respectively. Serum leptin correlation was studied with age, gender, BMI, waist, waist hip ratio, ALT, insulin resistance, class of NAFLD and hepatic necroinflammation and fibrosis.
Results: Patients with CHC had higher serum leptin levels in comparison to healthy controls (12.6±3.4 ng/ml vs.5.0±5.2 ng/ml, p=0.002) and NAFLD patients (12.6±3.4 ng/ml vs.5.9±3.3 ng/ml, p= 0.05). Serum leptin showed no correlation with age, gender, BMI, waist, waist hip ratio, ALT and insulin resistance in both the patient groups. There was no correlation of serum leptin with either class of NAFLD (NASH vs. no NASH) (p=0.75) and with grade of the disease and stage of hepatic fibrosis in both NAFLD (p=0.44) and CHC groups (p=0.59).
Conclusions: Patients with CHC have higher serum leptin levels in comparison to NAFLD. Serum leptin levels do not correlate with hepatic fibrosis in patients with NAFLD and CHC.

PP-240
Disseminated BCG infection with ascitis and hepatosplenomegaly, report of three cases in children
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Introduction: BCG vaccination at birth is done routinely in Iran during last 40 years.Adverse reactions induced by BCG vaccination are very rare (0-23.8%).291 cases of osteitis had been reported from 13 countries during 26 years (1948-1974). Disseminated infections are even rarer and their estimated incidence is 0.1-4.3 per million vaccinated children.
Materials & Methods: Three cases of BCGitis were hospitalized with poor condition. All of them had lymphadenopathy (LAP) with fistula to the skin in the injection site, abdominal mass, peritoneal and small intestine involvement.
Results: Fatal disseminated BCGitis is exceptional and affects specially the children with immune deficiency.
We report 3 cases of disseminated BCG infection leading to death in 2 cases. Two of them admitted with severe ascitis and multiple abdominal masses, they had got peritoneal and intestinal tuberculosis after BCG vaccination. The third one had hepatosplenomegaly, LAP and involvement of many organs including intestine and bones. All of them were immunedeficient.
Conclusion: Disseminated BCGitis is rarely seen after vaccination but is very dangerous and immunologic assessment often shows some defects.

PP-241
Oxysterols plasma levels correlate with chronic liver diseases
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Hepatic steatosis is a characteristic histological feature of patients with chronic hepatitis C. Several studies confirm the existence of a relationship between chronic hepatitis C and steatosis and suggest a direct role of the HCV core protein in inducing liver steatosis. Increasing experimental data suggest an important role of oxidative stress in chronic liver diseases. HCV infection is characterized by increased markers of oxidative stress, such as oxysterols, which could be involved in the evolution of liver damage. Oxysterols are sensitive

and specific markers of enhanced oxidative stress. Oxysterols bind to orphan nuclear receptors like Liver X Receptors (LXR) alpha and beta, that regulate expression of a number of proteins involved in cholesterol and fatty acid metabolism, including CYP7A and sterol regulatory binding element protein 1c (SREBP-1c). In addition, LXR-alpha controls the transcription of several genes involved in cellular cholesterol efflux including ATP-binding cassette (ABC)A1, ABC(G1) and apolipoprotein E. We investigate the relationship between HCV-related steatosis and oxysterol plasma levels, using a mass spectrometry assay to measure oxysterols (7-beta-hydroxycholesterol and 7-ketocholesterol) in patients with HCV infection and steatosis Patients with HBV infection and liver steatosis have been considered as controls. HCV infection causes a significant increase in plasma oxysterol concentration as compared with controls. Plasma oxysterol concentration correlates with liver ecogenicity and grading and staging of liver fibrosis (revealed by biopsies), in HCV-positive patients independently of sex, age and BMI.
This study provides new parameters to be considered when HCV patients undergo antiviral therapy.

PP-242
Clinical application of three-dimensional reconstruction of multi-slice computed tomography in the operation of liver neoplasm
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Objective: To evaluate the clinical value of three-dimensional (3D) reconstruction of multi-slice computed tomography (MSCT) in the operation of liver neoplasm.
Methods: Twenty-eight cases of liver neoplasm scheduled for resection were studied with MSCT by two-phase enhancement. The raw data images were processed on a workstation for 3D reconstruction. The findings after processing of the data were compared with those after surgery.
Result: High quality vascular images could be obtained by optimizing scanning technique and contrast agent administration. The 3D presentation could clearly display the vascular anatomy. Also, the 3D images could accurately depict and evaluate relationship of tumor to vessels such as pad effect, encasement, and involvement. This technique provides the surgeon with information about respectability and a "road map" for planning the liver neoplasm operation.
Conclusion: 3D reconstruction of MSCT can exhibit the accurate information of the liver neoplasm, and provide better surgical planning and management.

PP-243
A non-endoscopic predictor for esophageal varices in patients with liver cirrhosis
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Background/Aims: The aim of this study was to evaluate the clinical usefulness of a new non-endoscopic predictor of the presence of esophageal varices (EV) in cirrhotic patients.
Methods: Clinical data of 245 cirrhotic patients were retrospectively analyzed, who newly diagnosed (March 2002-July 2005), not previously bled and had a screening upper endoscopy. Platelet count x serum albumin/spleen size ratio [(PA/S ratio, (10^3/mm^3) x (g/dL)/cm] was assessed and the diagnostic accuracy was evaluated using area under ROC curves analysis. High risk varices for esophageal variceal hemorrhage were defined as medium-sized varices with red color signs or large varices.
Results: The main causes of liver cirrhosis were chronic alcoholism (42.4%), viral hepatitis (42.0%) and viral hepatitis/alcoholism (9.8%). The presence of EV and high risk varices were noted in 186 (75.9%) and 83 patients (35.9%), respectively. The AUROC of PA/S ratio for the presence of EV was 0.808 (95% CI, 0.746-0.869). PA/S ratio was significantly higher in patients with EV than without EV (28.9±18.5 vs. 57.1±31.9, p<0.001). The highest cutoff value of PA/S ratio was 36 with the sensitivity of 74.7% and positive predictive value (PPV) of 89.8% for the prediction of EV and was 29 with the sensitivity 80% and negative predictive value (NPV) 73.2% for the prediction of high risk varices.
Conclusions: PA/S ratio is a simple and useful predictor of the presence of EV in liver cirrhosis. Upper endoscopy should be recommended for the patient with the PA/S ratio less than 36, having the 90% probability of EV.

PP-244
Intrahepatic cholestasis of pregnancy: treatment with S-adenosyl-methionine plus heparin is superior to S-adenosyl-methionine alone
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Background: Heparin therapy for intrahepatic cholestasis of pregnancy (ICP) has never been reported. The purpose of this study is to compare the efficacy of s-adenosyl-methionine (SAM) plus heparin against SAM alone in the treatment of ICP.
Methods: A prospective, single-center, randomized control trial including 41 patients who were randomly by computer assigned to treatments. Each patient had a high level of cholyglycine, alanine-aminotranferase (ALT), aspartate-aminotransferase (AST) with or without pruritus and jaundice. Changes in biochemical indicators were analyzed with the paired *t* test.
Results: 16 patients received heparin plus SAM treatment with significant decrease of both cholyglycine and ALT (t=2.332, P <0.025; *t*=2.855, P<0.01, respectively). The platelet count and APTT monitor results no significant changes between post-therapy and pre-therapy. 25 patients received SAM alone treatment without a significant decrease of neither cholyglycine nor AST, only ALT (t=2.20, P<0.025). There was also a significantly shorter duration of treatment course in the heparin plus SAM group than that of in SAM alone. (*t*=3.417, P<0.005). There were no significant differences between the two groups with respect to gestational age at delivery and birth weight.
Conclusions: Heparin plus SAM provides a significantly faster improvement in biochemical indicators and a shorter treatment course than SAM alone for ICP.

PP-245
Intrahepatic cholestasis of pregnancy: Treatment with heparin alone is superior to S-adenosyl-methionine
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Background: Our previous studies showed that s-adenosyl-methionine (SAM) plus heparin treatment for intrahepatic cholestasis of pregnancy (ICP) was more efficacious than SAM alone. The purpose of this study was to evaluate whether heparin alone is superior to SAM.
Methods: A prospective, single-center trial includes 53 patients who were randomly assigned to treatments. Each patient had an elevated concentration of cholyglycine, ALT or AST, accompanied with or without pruritus and jaundice. The statistically significant differences in biochemical indicators between pretreatment and post-treatment with heparin or SAM, were analyzed with paired *t* test and Fish's exact *t* test or Wilcoxon signed-rank test if the data were didtribution-free.
Results: 28 patients received heparin therapy with significantly decrease of both cholyglycine and a-aminotranferase (cholyglycine: *t*=2.332, P <0.025; *t*=2.855, P<0.01, respectively). The platelet count and APTT monitor results no significant changes after therapy compared with before therapy. 25 patients received SAM alone treatment without significantly decrease of neither cholyglycine nor AST, only ALT, *t*=2.20, P<0.025). There was also significantly shorter of treatment course in heparin group than that of in SAM group (*t*=3.417, P<0.005). There were no significant differences between two groups with respect to maternal ages and admission ages of gestation.
Conclusions: Heparin alone treatment for ICP is superior to SAM with a significantly faster decrease of biochemical indicators and a significantly shorter therapeutic course without side effects.

PP-246
The relationship between elevated fibrinogen and the severity of intrahepatic cholestasis of pregnancy
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Objective: In clinical practice, we observed that most patients with intrahepatic cholestasis of pregnancy had elevated concentrations of plasma fibrinogen. The purpose of this study is to assess the relationship between those elevated concentrations of fibrinogen and the severity of the disease.
Methods: In this retrospective observational study, the plasma fibrinogen, alanine-aminotransferase, aspartate-aminotransferase and cholyglycine levels were analyzed from a total of 954 patients with intrahepatic cholestasis of pregnancy.

Results: 954 patients were reviewed. Fibrinogen concentrations are high with average of (5.455±1.46g/L). In Chi-Square analysis, the severity of the disease is strongly correlated with elevated fibrinogen (P<0.005). Different severity of the disease is impacted by different fibrinogen levels. The nature of the relationship is that patients with mild disease tend to have normal fibrinogen levels more frequently than that in severe patients, OR=2.10 (95% CI: 1.39-3.17); RR=1.41(95%CI: 1.16-1.78). Whereas the patients with severe disease tent to have the high fibrinogen concentration more frequently than that in others, OR=2.47 (95% CI: 1.35-4.52); RR=2.1 (95%CI: 1.18-3.42).
Conclusions: Elevated fibrinogen concentrations are closely correlated with the severity of intrahepatic cholestasis of pregnancy. These findings suggested that elevated plasma fibrinogen concentration might have a key role in the pathophysiology of this disease and there some important implications in the choosing of therapeutic approaches, especially, anticoagulant therapy.

PP-247
Nonalcoholic steatohepatitis induced by high fat diet can accelerate atherosclerosis
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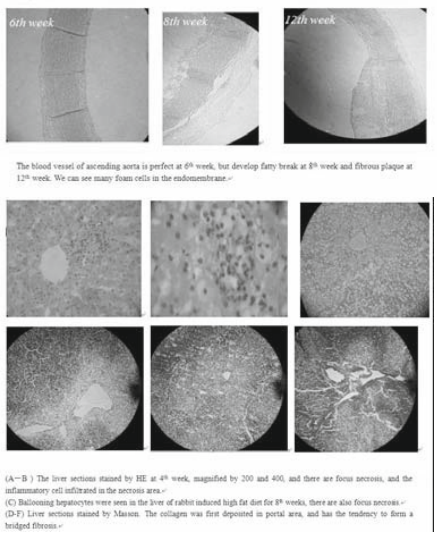
Background: Nonalcoholic steatohepatitis (NASH) is the progressing stage of nonalcoholic fatty liver disease, and NASH is strongly correlated with cardiovascular disease events independently of metabolic syndrome. But the existed NASH animal model induced by MCD diet is not insulin résistance, and can not reflect the effects of NASH on cardiovascular disease. This study was to establish a NASH model of rabbit induced by high fat, and to investigate whether the NASH is required in the pathogenesis in atherosclerosis.
Methods and results: The male New Zealand rabbit were treated with high fat diet which contains 10% corn oil and 1.5% cholesterol for 12weeks. The rabbit were sacrificed in batch at 2nd, 4th, 6th, 8th, 10th, and 12th weak at random. The rabbits already developed early NASH at 4th week, typical NASH at 8th week, early fibrosis at 12th week. However the arterial blood vessel only developed fatty break at 8th week and fibrous plaque at 12th week.

Conclusion: On the burden of lipid metabolism, the liver are the organ affected earlier than the great vessel, NASH can accelerated the atherosclerosis. And the exact mechanisms are needed to further investigate.



The blood vessel of ascending aorta is perfect at 6th week, but develop fatty break at 8th week and fibrous plaque at 12th week. We can see many foam cells in the endomembrane.

(A~B) The liver sections stained by HE at 4th week, magnified by 200 and 400, and there are focus necrosis, and the inflammatory cell infiltrated in the necrosis area.
(C) Ballooning hepatocytes were seen in the liver of rabbit induced high fat diet for 8th weeks, there are also focus necrosis.
(D-F) Liver sections stained by Masson. The collagen was first deposited in portal area, and has the tendency to form a bridged fibrosis.

PP-248
Prediction of esophageal varices in children
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Background: The identification of children at greatest risk of having esophageal varices (EV) using non-invasive tests would facilitate selection of children for future studies of primary prophylaxis of variceal hemorrhage.
Aim: To measure the ability of non-invasive tests to predict the presence of EV in children.
Methods: In this retrospective study, consecutive children <18y with liver disease who underwent endoscopy between 2000 and 2006 were identified.
Results: 51 children were eligible for inclusion. EV were found in 17 of 51 patients. Parameters found to differ significantly between children with and without varices included platelet/spleen size ratio (p<0.001), platelet count (p<0.001), INR (p=0.001), AST/ALT ratio (p=0.002) and albumin (p=0.003); their diagnostic accuracy was assessed by the measures shown in the table.
Conclusions: Our results suggest that is possible to identify children with EV before undertaking endoscopy, and will be validated by a prospective study.

Parameter	Cut-off v	Sensitivity	Specificity	PPV	NPV	LR+	LR-	AUROC
Pl/Spl size ratio	148	94	73	0.71	0.94	3.44	0.09	0.91
Platelet count	136 x10^9	81	96	0.93	0.9	23	0.18	0.88
INR	1.08	76	67	0.59	0.83	2.38	0.35	0.79
AST/ALT ratio	1.12	71	68	0.57	0.8	2.27	0.43	0.79
Albumin	41.5	77	67	0.59	0.83	2.38	0.35	0.77

PP-249
The role of serum pro-BNP natriuretic peptide in the differential diagnosis of ascites due to heart failure
Lambrini Skorda[1], Nikitas Papantoniou[1], Ioannis Mathaios[1], Alexandros Kotis[1], Philippos Lisgos[1], Aggelos Delaportas[1], Elias Tserkis[1], Stylianos Karatapanis[1]
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Background and Aims: The differential diagnosis of ascites due to cirrhosis from the ascites due to heart failure may be difficult using only clinical criteria. Usually it is necessary to use more invasive test including paracentesis with measurement the serum-ascites albumin gradient and in some cases the measurement of hepatic venous pressure gradient. Brain-type natriuretic peptide (BNP) and its inactive pro-hormone (pro-BNP) have been proved useful parameters in the management of patients with heart failure. Therefore the aim of the present study was to assess the role of serum pro-BNP in the differential diagnosis of ascites due to cirrhosis from ascites due to heart failure.
Methods: In the present study there were included 77 patients (M/F= 52/25, mean age 53) with ascites due to cirrhosis and 15 patients with ascites due to heart failure. The aetiology of cirrhosis was HBV=30, HCV=28, Alc=14, misc=6. In all patients with ascites we measured serum pro-BNP levels.
Results: The median serum pro-BNP levels in the patients with ascites due to heart failure were significantly higher (5870 pg/ml, range=1210-17300) as compared with those observed in the cirrhotic patients (156.6 pg/ml, range=37-1688) (P<0.001).
Conclusion: The results of our study showed that serum pro-BNP measurement could be a useful and powerful marker in the differential diagnosis of ascites due to cirrhosis from ascites due to heart failure.

PP-250
M2-3E autoantigenes evaluation of measurement of M 2 autoantibodies in diagnosis of primary billiary cirrhosis
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Objective: To detect the value of measurement of M2 autoantibodies in diagnosis of primary biliary cirrhosis (PBC).
Methods: Serum from 96 patients with primiary biliary cirrhosis,a control panel of 100 patients with other autoimmune disease and 50 healthy blood donors were analysed using the Anti-M2 ELISA, Anti-BPO ELISA,Anti-M2-3E ELISA.
Results: The sensitivity of the Anti-M2 ELISA for PBC was 81.25%,with a specificity of 97.15%;The sensitivity of the Anti-BPO ELISA for PBC was 88.54%,with a specificity of 98.37%;The sensitivity of the Anti-M2-3E（BPO）ELISA for PBC was 96.88%,with a specificity of 90.24%.
Conclusion: The sensitivity of the Anti-M2-3E（BPO）ELISA for PBC was best,with a high specificity,It should be used as a powerful and specific maker for the diagnosis of PBC ·

PP-251
The clinical significance and detection of autoantibody repertoire in primary biliary cirrhosis
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Objective: To detect the value of measurement of autoantibody repertoire in diagnosis of primary biliary cirrhosis.

Methods: Detecting Serum samples AMA-M2,3E (BPO) ,Sp100,PML,gp210, SLA/LP,96 patients with PBC and 100 patients with other autoimmune disease and 50 healthy blood donors by EOROLINE.

Results: The sensitivity of the Anti-M2 for PBC was 76.0%, with a specificity of 90.7%; The sensitivity of the Anti-BPO for PBC was 84. 4%,with a specificity of 94.0%;The sensitivity of the Anti-Sp100 for PBC was 12.5%,with a specificity of 97.3%;The sensitivity of the Anti-PML for PBC was 28.1%,with a specificity of 79.3%;The sensitivity of the Anti-gp210 for PBC was 51. 0%, with a specificity of 93.3%; The sensitivity of the Anti-SLA/LP for PBC was 21. 9%,with a specificity of 74.7% .

Conclusion: The test of autoantibodies is clinically significant for the diagnosis of PBC, autoantibody repertoire may be helpful for therapy and prognosis of PBC.

PP-252

Increased killing of liver NK cells contributes to MHV-3 induced fulminant hepatic failure in Balb/cJ mice

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To investigate the role of liver NK cells in the development of hepatocytes necrosis and fulminant hepatic failure (FHF) induced by MHV-3. Balb/cJ mice (6-8 weeks, female) were intraperitoneally injected with 100 PFU MHV-3.The ratios and absolute numbers of NK cells in liver, blood, spleen and bone marrow and the CD69 expression of liver NK cells at 0h, 24h, 48h and 72h post MHV-3 infection were analyzed by flow cytometry. The cytotoxic activity of liver NK cells and the level of IFN-γ produced by liver NK cells at oh and 48h post infection were measured by a non-radioactive cytotoxicity assay and intracellular cytokine staining, respectively. Following MHV-3 infection, the proportion of NK cells in liver and peripheral blood in Balb/cJ mice increased remarkably and peaked at 48h post infection, then kept at a high level in liver until the mice sacrificed. The CD69 expression on liver NK cell was highly up-regulated and the cytotoxic activity was also significantly enhanced with an increasing in intracellular IFN-γ production at 48h post infection. Whereas in spleen and bone marrow, the proportions of NK cells were both significantly decreased from oh to 48h and then slightly increased. Our data demonstrated that the recruitment of NK cells, probably from spleen and bone marrow, and their activation in liver correlated with hepatocyte necrosis and disease severity in Balb/cJ mice infected with MHV-3 ，indicating the provital involvement of NK cells in pathogenesis of FHF. This study was supported by (No.3057164, No.30672380) and (2007CB512900, 2005CB522901)

PP-253

Fatty liver disease in correlation to computer tomography scan density measurement

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Background and aim: Liver steatosis is a clinical-pathological condition that only recently has been recognized to be a common and potentially progressive to severe form of liver disease. Our aim was to evaluate the causes of isolated elevation of gamma glutamil transpeptidase (GGT) and correlation with clinical and imagistic investigation: ECHO and CT scan density measurement of liver and spleen attenuation.

Material and method: We studied 83 patients with elevated GGT by biochemical, imagistic (ECHO, CT) and functional biochemical liver tests. We searched additional contributing factors: drugs, alcohol intake.

Results: All had hepatic steatosis, 43 have elevated TGP, 8 patients had HVC chronic infection, 9 had markers of chronic Cytomegalovirus infection associated with and recent drugs consuming (antibiotics, AINS), 18 moderate alcohol intake, 23 chronic drug consuming (antibiotics, AINS, benzodiazepines). Elevation of TGO, TGP was present in associated contributing factors: hepatotoxic drugs, viral infections and alcohol. . The presence of hepatic steatosis was significantly correlated with total abdominal fat area and visceral fat area. There was a significant difference in the craniocaudal liver span between patients with NASH (mean 19cm) and patients with steatosis (mean 15cm) ($P<0.01$).

Conclusions: Liver-to-spleen CT attenuation ratio (L/S) of less than 1, indicating hepatic steatosis. Elevated persistent GGT is a biochemical sign of mild-severe steatosis.

PP-254

Does PNALT(persistently normal ALT) predict HBVDNA activity in e-antigen negative CHB(chronic Hepatitis B) patients?

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Background: E-antigen negative CHB infection is common in Malaysia. High viral load (>10,000copies/ml) may be associated with pre-C/BCP gene mutation(s) and e-antigen negative disease.

Objective: To determine the percentage of our HBeAG-vePNALT CHB patients with viral load >10,000copies/ml.

Methods: The records of the last 100consecutive PNALT HBeAG-ve treatment-naïve CHB patients were reviewed. All HBVDNA assay (RocheCobasAmpliprep/Tagman) were sent to Malaysia Liver Foundation. Patients with CHC, HIV or decompensated cirrhosis were excluded. Upper limit normal of ALT (U/l) for male and female is 43 and 33 respectively.

Results: 100patients were followed-up for median of 28.5months (range=12-84) and all patients had a normal ALT for median of 6 occasions(range=3-40) before HBVDNA assay.

The median age were 47 years (range: 17-72) with male gender predominance over female at ratio of 6:4. The Chinese, Malay, Indian racial composition was 7:2:1.

74% of our patients did not have any abnormal ALT reading. The median maximum ALT was 25U/ml(range:11-43).

The median maximum ALT for the remaining 36% of patients who have had abnormal maximum ALT was 53U/l(range:34-1129) with the last abnormal ALT being at median of 33months(range:13-40) before HBVDNA assay.

10,25%,40% and 25 % of our patients had HBVDNA of > 100000 ,>10000-99999,>320-10000 and ≤320 copies/ml respectively.

Conclusion: 35% of our CHB patients who are e-antigen negative have high HBVDNA>10,000copies/ml despite PNALT. Hence, HBV DNA assay should be done in all PNALT HBeAG-ve patients to identify patients at risk of e-antigen negative disease who may need treatment.

PP-255

Lateral dissection technique: towards safer laparoscopic cholecystectomy

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Introduction: Laparoscopic Cholecystectomy (LC) is accepted worldwide. The aim of this study was to evaluate the efficacy and safety of lateral dissection technique during LC.

Methods: Between October 1995 and September 2005. 1645 LC were performed at the National Liver Institute and Mahmoud Hospital adopting “lateral dissection technique”. Dissection starts from a safety zone at the lateral edge of gall bladder neck .Close to gall bladder wall, the peritoneum at the lateral side of the Hartman pouch is opened from above down to the junction with the cystic duct. Gall bladder base is freed from liver bed until a window above the hepatic pedicle is opened. With minimal dissection the cystic artery and duct are clipped.

Results: 989 females and 656 males with mean age of 43 years were included. Hospital stay ranged from 1-12 days.Four cases (0.24%) were converted to open technique (2 for adhesions and 2 for bleeding). 279 cases (16.9%) had acute cholecystitis. Intraopertaive cholangiography was performed in 213 (12.9%).Mortality was nil. Eight cases (0.48%) developed bile leak (5 cystic duct stump and 3 accessory liver bed duct). Three were managed conservatively and 3 by endoscopic stenting .Surgery was necessary in 2 cases. Missed CBD stones detected in 4 cases (0.24%); all successfully treated endoscopically. There were no bile duct injuries.Port site hernia occurred in 11 cases (0.66%), while wound infection in 18 cases (1.09%).

Conclusion: Improving safety of LC can be achieved by dissection of the zone lateral to edge of gall bladder neck with minimum dissection of Calot's triangle.

PP-256

Systematic bioinformatics mining of transcriptome and proteome data in metastatic hepatocellular carcinoma research

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Mapping the network that give rise to metastasis is one of the key challenges of hepatocellular carcinoma research. Here, we apply a systems biology based approach to identify models correlated with metastasis not as individual genes but as subnetworks. After systematic experimental design and large scale profiling of transcriptome and proteome in metastatic hepatocellular carcinoma cell lines (MHCC97L, MHCC97H, HCCLM3 and HCCLM6), serials of bioinformatics (mathematics) artifices have been carried out to seek hidden clues associated with metastasis mechanism: (1) biological function enrichment analysis based on k-mean clustering of transcriptome and proteome data; (2) pathway node analysis combining with transcriptome expression patterns; (3) pathway topology, chromosome cytoband distribution and interaction analyses under transcriptome co-expression patterns; (4) quantitative integration of transcriptome and proteome data; (5) globe transcriptome comparison among the 4 metastatic hepatocellular carcinoma cell lines and other references (Hep3B, hepatocyte, normal liver tissue and MCF7). These analyses have revealed a wealth of discoveries related to lung, lymphatic and collectivity metastasis abilities respectively. Most of them are in good agreement with previous knowledge and some have never been reported before. Further experimental validations and perturbations have been designed based on these results.

PP-257

Small intestinal bacterial overgrowth of colonic-type carbohidrates fermentative bacteria in cirrhotic patients

Giuseppe Merra[1,2], Antonio Dal Lago[1,2], Emidio Scarpellini[1,2], Rosalba Finizio[1,2], Michele Santoro[1,2], Maria Grazia Spitilli[1,3], Ernesto Cristiano Lauritano[1,2], Maurizio Gabrielli[1,2], Venanzio Valenza[1,3], Giovanni Gasbarrini[1,2], Giovanni Ghirlanda[1,2], Antonio Gasbarrini[1,2]
[1] *Catholic University of Sacred Hearth - "A. Gemelli" General Hospital, Rome,* [2] *Dpartment of Internal Medicine ,* [3] *Department of Nuclear Medicine*
Backgrounds and Aim: Small intestinal bacterial overgrowth (SIBO) is a clinical condition characterized by abnormally high colonic-type bacteria in the small intestine, exceeding 10^6 organisms/mL. In non cirrhotic patients, SIBO is associated with the presence of symptoms related to malabsorption and gas production (end product of carbohydrates fermentation).. A role of small bowel bacteria has been hypothesized in the pathogenesis of hepatic encephalopathy (HE) and spontaneous bacterial peritonitis (SBP) in cirrhotics. This study to assess SIBO prevalence in cirrhotic patients.
Methods: thirthy (30) HCV-cirrhotic pts (10 Child A; 10 Child B; 10 Child C) were consecutively enrolled and submitted to H2-lactulose breath test (LBT). 30 non cirrhotics patients were used as controls. SIBO diagnosis was based on LBT positivity criteria (two distinct peaks, consisting of two consecutive H2 values >10 p.p.m. above the basal value after 10 g lactulose ingestion).
Results: 18 out of 30 cirrhotics (60%) had a positive LBT vs 1 out of 30 controls (3.3%); $p<0.05$. Among cirrhotics, a significative difference was observed in the different Child group: 20% in Child A, 50% in Child B, 80% in Child C.
Conclusion: Cirrhotics have a significant prevalence of SIBO compared to controls. SIBO prevalence was associated to severity of cirrhosis. Lactulose administration could be a good substrate for the growth in the small bowel of fermentative colonic-type bacteria. A role of SIBO presence in HE and SBP has to be fully evaluated.

PP-258

Effect of the novel surgical procedure -selective decongestive devascularization shunt of gastrosplenic region (SDDS-GSR) for treatment of portal hypertension

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Background: Up to now, there are no satisfying surgical procedures for the treatment of portal hypertension of liver cirrhosis. After current surgical procedures, the rate of variceal rebleeding and hepatic encephalopathy remain still relatively high or hypersplenism can not been improved efficiently. To improve the therapeutic effect of surgical procedures, we designed the novel surgical procedure -selective decongestive devascularization shunt of gastrosplenic region (SDDS-GSR).
Methods: SDDS-GSR was performed in 30 cirrhosis patients with portal hypertension and all the patients were followed up for 3-36 months. Compared with preoperative conditions and 20 devascularization operations, the portal pressure, diameter of portal vein, and size of spleen were recorded. The change in peripheral blood cell counts, serum levels of IL-6, sIL-2R and liver function were observed.
Results: SDDS-GSR could decompress the venous system in the gastroesphageal area ($P<0.01$) while maintain portal perfusion. The size of spleen was significantly reduced ($P<0.01$) and the hypersplenism improved. After the operation, there were no hepatic encephalopathy and variceal rebleeding. Compared with non-operation group, the levels of IL-6, sIL-2R were significantly reduced to (2.11±0.59) pg/ml and (124.98±36.93) pg/ml in SDDS-GSR group ($P<0.05$).
Conclusions: SDDS-GSR is a reasonable and reliable surgical procedure for portal hypertension causing esophageal and gastric varices.

PP-259

Globe gene expression changes induced by NOR1 over-expression in HepG2 cells

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Previous work from laboratory has cloned a novel gene NOR1 and showed its extensive expression in normal tissues and down-regulation in carcinomas. To further investigate its downstream target genes and better understand its function, NOR1 over-expressed HepG2 hepatoma cell line was established by gene transfection, and global changes in gene expressions were identified by cDNA microarrays. The results discovered 59 genes up-regulated in these cells compared with the original cells, including Grb2, HBP17, TNFRSF11B genes that have been implicated in numerous roles in tumorigenesis and cancer development. In addition, 103 down-regulated genes were also identified, including genes encoding Bik, MAP2K6 and ZFP95 proteins. The expression patterns of certain genes identified by microarrays were validated by quantitative real-time PCR and showed statistics significance difference ($p<0.05$).The results indicated that consistent results could obtained from above two methods. These data suggest that NOR1 may influence the biology and cancerous behaviors of HepG2 cells by exerting its effects on the expression of a set of genes involved in cell signal transduction, cell cycle regulation, transcription regulation and translation control related genes.

PP-260

Epidemiologic features of nonalcoholic fatty liver

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Background and Aim: Nonalcoholic fatty liver disease (NAFLD) is a common condition in Western. The aim of this study was to determine the prevalence of NAFLD which was diagnosed by ultrasonography and the risk factors in an Eastern country (in Turkish general population which is a country between Asia and Europe).
Patients and methods: A hepatobiliary ultrasonography was performed on 459 subjects selected randomly from the general population in Elazig provinence. BMI (Body Mass Index) and waist circumference were calculated; plasma lipids, glucose, aminotransferases levels, and viral serology were evaluated.
Statistical Analysis: chi-square, t-test and logistic regression were all used.
Results: 408 subjects; age: range from 18 to 80 years included. Four subjects were excluded due to the regular alcohol consumption in 1 subject and chronic viral hepatitis in 3. Of the 404, the prevalence of NAFLD was 19.8%; and 16.5% female and 23.7% were male (%females vs. %males, $p < 0.05$). NAFLD was the most prevalent in the fifth decade and more common in males than females under 40 years age group ($p <0.05$). There was a significant association between NAFLD and BMI, waist circumference, serum ALT and triglyceride levels. Logistic regression analysis revealed that waist circumference and advanced agein females while waist circumference and increased ALT in males were independent predictors for NAFLD.
Conclusion: NAFLD is a common disease in Turkey as well as in Western. NAFLD affects one-fifth of the Turkish population and more common in young males. Central obesity appears an indipendent factor for the development of NAFLD.

PP-261

A study on intra-species genotipical variants of fasciola hepatica in various animal hosts using RAPD-PCR

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Liver fluke disease or fasciolasis is one of the most important parasitic diseases throughout the world. We compared genetic pattern between four species from different hosts, both intra-species and inter-species. We studied 53 worms of *Fasciola hepatica* obtained from bile ducts of naturally infected cattle, sheep, buffalo and goat during nine months. Genomic DNA was extracted from 70% ethanol fixed flukes. RAPD-PCR with a set of arbitrary primers (A and B) used to describe genetic variation and estimation of genetic diversity in endemic regions of Iran. Some samples went under sequencing determination. ITS1 region was amplified successfully for all samples and in all cases showed a band of 460 bp. Our result showed significant genetic variations in ITS1 region based on a 460 bp PCR product coming from primer A. the pattern of RAPD-PCR in all isolates belong to different hosts and also in isolates form the same host demonstrated differences so that sheep samples divided to three categories, cattle's to two and buffalo's to two categories. ITS1 region showed a similar quantitative weight and its sequencing approved this issue. ITS1 region in this parasite is highly conserved. We found that Iranian samples were similar to foreign samples as 100% and only 2% with *F. gigantica.* Considering the fixation of the sequences in this region it is probable that using RFLP to differentiate between *F.hepatica* and *F. gigantica*. This assay is useful for Phylogenic, Taxonomic and Molecular Epidemiologic studies of organisms and specially micro-organisms.

PP-262

Transcatheter obliteration of porto-systemic shunt in hepatic encephalopathy

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Objectives: To evaluated the efficacy of transcathter obliteration of porto-systemic shunt in hepatic encephalopathy.

Methods: 2 cases of recurrent hepatic encephalopathy patients due to spontaneous gastro-renal or spleno-renal shunt were treated with transcatheter obliteration of porto-systemic shunt resparetely, balloon-occluded retrograde transvenous obliteration or percutaneous transhepatic obliteration.

Results: The serum ammonia level of both patients were declined to normal range several months after procedure and there were no neuropsychic symptoms in the follow-up periods.

Conclusions: The transcatheter obliteration of spontaneous gastro-renal or spleno-renal shunt was effective in porto-systemic shunt hepatic encephalopathy, but more cases and longer follow-up period were needed to investigate the therapeutic effect and complication.

PP-263

Clincal investigation on the relations of leptin, insulin and BMI in patients with NAFLD

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A**ims:** NAFLD clinical pathological characteristics are similar to those of alcoholic liver diseases, and might progress forward serious diseases. This study aims to investigate the correlations between BMI, glucose, lipid profile, AST, ALT, leptin, insulin , insulin sensitivity in patients with NAFLD.

Methods: 31 cases of patients with NAFLD and 30 cases normal control were divided into two groups according to their BMI: BMI < 28kg/m^2 group and BMI≥28kg/m^2 group. The height , weight, waist circumference,hip circumference, glucose,TC, TG, ALT, AST, insulin, leptin were measured . T test, F test, Sample, multiple stepwise regression analysis of correlationship were used.

Results: Insulin in patients with NAFLD were significantly higher than thatcontrols（$p<0.01$）.Insulin of obese NAFLD were significantly higher than that normal BMI ($p<0.01$). Leptin in patients with NAFLD were not different from the controls($p>0.05$). Sample analysis of correlationship showed that the height, BMI, waist circumference are correlated with serum leptin level.Multiple stepwise regression analysis showed that the waist circumference, waist-hip ratio, insulin are positively correlated leptin level. Height, weight, AST/ALT, are negatively correlated with leptin. BMI，FPG，AST，are negatively correlated with ISI.TG is positively correlated with ISI.

Conclusion: Patients with NAFLD, there is obvious insulin resistance.Discrepancy in insulin resistance was also found among different BMI patients.Distribution of body fat and insulin resistance, but not serum cholesterol and triglyceride, are closed correlated with fasting leptin level.

PP-264

Management of Bleeding Duodenal Varices in cirrhosis

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Background/aims: Duodenal varices (DV) are a rare site of hemorrhage in cirrhotic portal hypertensive patients, but their rupture is a serious and often fatal event. Being rare, experience in the control of hemorrhage from DV is also limited. Consequently, there is no consensus as to the best treatment of bleeding DV in cirrhosis. Therefore, this study was conducted to evaluate the efficacy and safety of endoscopic injection therapy (EIT) with n-butyl-2- cyanoacrylate in the treatment of 10 patients with upper gastrointestinal (UGI) bleeding due to ruptured DV.

Methods: emergency UGI endoscopy was done within 24 hours of admission and EIT was then undertaken by injecting one or 2 cc of cyanoacrylate containing 30% lipidol strictly intravariceally to stop bleeding. The patients were placed on acid suppression and abdominal radiography was done for every patient 24 hours after EIT to trace the injected material inside the occluded varix.

Results: successful hemostasis was achieved in all patients (100%) with no further bleeding episodes during the 6 months of follow-up. No serious complications were observed except for a small postsclerotherapy ulcer in 3 patients (30%) endoscopic examination at the end of follow-up period revealed disappearance of DV in 8 patients (80%).

Conclusion: the experience reported in the present study confirms the efficacy and safety of this hemostatic modality in the management of this rare but often fatal cause of UGI bleeding in cirrhosis.

PP-265

Cholesterol metabolism in cultured steatotic hepatocytes and the effect of adiponectin on it

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Objective: To explore cholesterol metabolism and expression of message RNA of relevant genes in cholesterol synthesis in the models of cultured steatotic hepatocytes, and the effect of adiponectin on them.

Methods: Steatosis model of hepatocytes was established by adding palmic acid to the growing L-02 cells. The cells were divided into three groups: the first group added palmitic acid for three days then gave RPMI-1640 culture solution without palmitic acid for 24 hours; the second group added palmitic acid for three days then gave adiponectin in RPMI-1640 culture solution for 24 hours; the third added only RPMI-1640 culture solution for four days. The intracellular triglyceride and total cholesterol were detected by using analyzed kit. The expression of SREBP-2 and its target gene hydroxymethylglutaryl CoA reductase (HMGCR) was measured with RT-PCR.

Results: At the third day, hepatocyte steatosis was observed, and the contents of intracellular triglyceride and cholesterol increased, the expression of SREBP-2 and HMGCR mRNA was up-regulated at the first and second group than the third group. At the fourth day, the contents of intracellular triglyceride and cholesterol reduced the expression of SREBP-2 and HMGCR mRNA was down-regulated at the second group compared with the first group, but still higher than the third group.

Conclusion: There is a cholesterol accumulation as a result of increased expression of relevant genes in cholesterol synthesis in steatotic hepatocytes. Adiponectin can reduce both the content of intracellular triglyceride and cholesterol and SREBP-2、HMGCR mRNA expression in cultured steatotic hepatocytes.

PP-266

Ultrasonography in biliary ducts atresia

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Background: In this study, we evaluated and compared two different diagnostic methods, ultrasonography and HIDA scan for this purpose.

Methods & Materials: Sixty infants with cholestatic jaundice were studied in a prospective study during a 2-year period. The results of liver biopsy considered as the definite diagnosis (gold standard).
Results: Sixty infants, 35 males and 25 females, with the mean age of 56.1±17.8 days were enrolled in the study. According to the results of liver biopsy, there were 16 (26.7%) cases of EHBA, and 24 (40%) cases of hepatitis. Ultrasonography detected EHBA in 15 cases and HIDA scan diagnosed it in 38 patients. Sensitivity, specificity, positive predictive values, negative predictive value and accuracy of ultrasonography and HIDA scan in diagnosis of EHBA were 87.5%, 97.7%, 93.3%, 95.7%, 95%; and 100%, 50%, 42.1%, 100% and 63.3%, respectively. Four patients died during follow-up. None of the expired patients belonged to EHBA group.
Conclusion: This study showed a higher specificity and accuracy of ultrasonography for diagnosis of EHBA. It is recommended to consider ultrasonography in the first step of diagnosis in patients with suspected EHBA.

PP-267
Cystatin C in Chronic Liver Diseases
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Serum cystatin C concentration is closely related to the degrees of fibrosis determined histologically and is significantly elevated even in patients with mild hepatic fibrosis.
Aim: to study the applicability of serum cystatin C as a marker in monitoring the progression of chronic liver diseases.
M/M: included 74 male patients with chronic liver diseases of different grade of severity with an age range of 20-63 ys subdivided into: *Group I*: 27 patients with liver cirrhosis with different Child-Pugh grade [Group Ia: Child B cirrhotic patients (n=11) & Group Ib: Child C cirrhotic patients (n=16)]. *Group II*: 47 chronic hepatitis C patients, they subdivided into [Group IIa: Chronic HCV infection patients with normal ALT value (n=24) &Group IIb: Chronic hepatitis C patients with raised ALT value (n=23)]. *Group III* 25 healthy controls with an age range of 18-55 years.
Results: Serum cystatin C level was significantly higher in group Ia, group Ib and group IIb as compared to the control group and in group IIb as compared to group IIa ($p<0.001$). No significant difference between group IIa as compared to the control group and group Ia as compared to group Ib ($p>0.05$).Also,there was no significant correlation between cystatin C and age, platelet count or ALT in groups I, Ia, Ib, IIa and IIb. No significant correlation between cystatin C and age and platelet count in group II, while there was a positive correlation between cystatin C and ALT value. No relation between cystatin C concentrations and the process of necro-inflammation was detected.
Conclusions: Serum cystatin C increased with increased severity of liver disease, but its applicability as a marker for liver disease progression is still questionable. Cystatin C couldn't differentiate between cirrhotic patients with different

PP-268
Quantitative evaluation of liver function by the 13C-Methacetin Breath Test in healthy volunteers and the liver disease
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Background: To grade liver damger, Child-Pugh classification is used but the test do not replect the quantitative functional hepatic reserve.
Aim: ^{13}C-Methacetin Breath Test are evaluated as possible tool, being both safe and easy to perform to quantify hepatic reserve in chronic liver disease patients.
Methods: Breath test was performed in 30 healthy volunteers and 50 chronic liver disease patients.Breath samples were collected after taking ^{13}C-Methacetin (75mg). $^{13}CO_2$ enrichment was measured using Nondispersive Intrared Isotope Analyser (WAGNER).
Results: There are significant difference between the heathy volunteer and chronic hepatitis, liver cirrhosis classifing Child-Pugh A, liver cirrhosis classifing Child-Pugh B/C in delta over baseline (DOB), and in the cumulative $^{13}CO_2$ excretion (Cum.dose) too ($P<0.05$).A significant difference for the cumulative $^{13}CO_2$ excretion (Cum.dose) was obersed among different cirrhosis stage(Child-Pugh A→Child-Pugh B/C) ($P<0.05$).
Conclusion: ^{13}C-Methacetin Breath Test are safe and easy tests to perform and is able to discrimnate the hepatic functional capicity between the different groups studied.

PP-269
Role of insulin resistance in etiopathogenesis of non-alcoholic fatty liver disease
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Introduction: The non-alcoholic fatty liver disease (NAFLD) is characterized by fatty changes in the liver. The causes are not yet well known, and considered of multifactorial origin. Many authors consider that the NAFLD is a manifestation of metabolic-syndrome and that the insulin resistance is an etiopathological factor.
Objective: To identify a group of patients with NAFLD the existence and degree of insulin resistance, and to compare them with a control group of patients without liver disease.
Patients and Methods: We studied 109 non-diabetic patients; 65(51 men and 15 women) with prior diagnosis of NASH, with average age of 49.3(± 11.9) years and 44 (29 men / 15 women) without liver pathology, with an average age of 54.8(±13.38) years.
In all patients we measured fasting plasma glucose levels, basal plasma insulin and HOMA-IR. We evaluated and compared patients with HOMA-IR>2.5 between and we calculate the statistical significance using the t-Student test.
Results:
1.- Patients with NAFLD:
Fasting plasma glucose average: 107.40(±12.97)mg/dl.
Plasma insulin average: 14.07(±7.15)µUI/ml.
HOMA-IR average: 3.78(±1.97).
Number of patients with HOMA-IR> 2.5: 48/65(73.84%)
2.- Patients without NAFLD:
Fasting plasma glucose average: 107.70(±12.80)mg/dl.
Plasma insulin average: 7.58(±2.88)µUI/ml.
HOMA-IR average: 2.00(±0.75).
Number of patients with HOMA-IR> 2.5: 11/44(25%).
3.- Comparison of the two groups with the t-Student test:
t-Student: 5.48
P-value: 0.0001
Conclusions: The insulin resistance is present in much of patients with NAFLD (73.84%). Both hepatic steatosis and fibrosis are the result of numerous factors but it is clear that the state of insulin resistance plays a critical role in the pathogenesis of NAFLD.

PP-270
Investigation of biliary biochemical constituents and cytokines in infantile hepatitis syndrome
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This study is to investigate the biliary biochemical constituents and cytokines in infantile hepatitis syndrome (IHS). As results, In cholestasis group, the value of serumal TBIL, DBIL, ALT, γ-GT, TBA, IL-6 and TNF-α was higher than that of control ($p<0.01$); the value of biliary TBIL, DBIL, γ-GT and TBA was lower than that of control, and that of biliary IL-6 and TNF-α was higher than that of control ($p<0.01$). In cholestasis group, the value of IL-6 and TNF-α in serum was lower than that in bile ($p<0.01$). In hepatitis group, the value of serumal DBIL, ALT, γ-GT, TBA, IL-6 and TNF-α was higher than that of control ($p<0.01$ or $p<0.05$); the value of biliary TBIL, DBIL, γ-GT and TBA was lower than that of control ($p<0.01$), and that of biliary IL-6 and TNF-α was higher than that of control ($p<0.01$). In hepatitis group, the value of IL-6 and TNF-α in serum was also lower than that in bile ($p<0.01$). The value of serumal TBIL, DBIL, γ-GT, IL-6 and TNF-α in cholestasis group was higher than that in hepatitis group. The value of biliary IL-6 and TNF-α was correlated to that of serumal DBIL, TBA and γ-GT in IHS subjects. In conclusion, Biliary biochemical constituents altered coincidence with pathological changes in hepatocellular injury. Cholestasis is more serious in subtype of cholestasis in IHS patients. Assay of biliary IL-6 and TNF-α can be a specific and sensitive reference to determine the inflammation status of the impaired liver in IHS.

PP-271
Oxysterols sensitize hepatic cells to transformation
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Experimental data suggest a role of oxidative stress (OS) in hepatocellular carcinoma (HCC) induction. Very high plasma levels of oxysterols, 7-betahydroxy-cholesterol and 7-ketocholesterol, have been

found in patients with end-stage liver cirrhosis. They regulate gene expression and cholesterol homeostasis and carcinogenesis. The purpose of this study was to investigate 7-betahydroxy-cholesterol, 7-ketocholesterol and 5,6-secosterol effects in cell homeostasis. HepG2 cells were treated with different amounts of oxysterols for early and late time points. We observed that treatment of HepG2 cells with 1nM, 10 nM of oxysterols weakly induced cell growth; whereas 100 nM and 10 induced a significant decrease in cell number (at 72h). The FACS analysis has revealed that HepG2 cells accumulated in G0/G1 phase; a weak increase of cells number were observed in G2/M phase, too. Interestingly, treatment of HepG2 cells with 100nM of oxysterols induced an increase of ERK1/2 activity at 1h and its down-regulation at late time points. JNK activity, on the contrary, didn't change at early time points; whereas at late time points, JNK expression and activity were up-regulated by low dosages (1 nM and 10 nM) and down-regulated by high dosages (100 nM and 10 nM). P38MAPK activity increased at 30' with 1 nM, 10nM and 100 nM of oxysterol concentrations; instead at 1h we observed a down-regulation.
The data reported above suggest that oxysterols modulate and interfere with hepatic cell homeostasis, sensitizing cells to transfomation. So it could be relevant to identify new predictive markers of liver transformation and for targeting new therapies.

PP-272

Ultrastructural changes of canal of Hering in chronic liver disease represents hepatobiliary dysfunction

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Background: A network of bile canaliculi drains into the intrahepatic ductule, called canal of Hering, located in hepatobiliary junction, and drains into the bile ductule. The canals are partially lined by hepatocytes on one aspect and by ductular cells on the other. In this study, ultrastructural changes of canal of Hering caused by chronic liver disease was investigated.
Methods: We have conducted electron microscopy, immunohistochemistry and polymerase chain reaction (PCR) in five normal liver tissues and tissues from thirteen patients with chronic hepatitis B, six patients with chronic hepatitis C, fifteen patients with liver cirrhosis.
Results: Staining with carcinoembryonic antigen (CEA) revealed transition from bile canaliculi to intrahepatic ductules. The bile canaliculi were elongated, distended, and arborescent. The mean area of dilated bile canaliculi in chronic hepatitis B was largest, and that was larger in chronic liver disease than in normal liver (p<0.05). The increased expression of cross reactive to biliary glycoprotein (BGP) mRNA in the tissues from chronic liver disease was shown by PCR. Double immunohistochemistry with HepPar1 for hepatocyte and CK 7 for biliary epithelial cell confirmed canals of Hering. In electron microscopy, hepatocytes and ductular cells composing canals of Hering were degenerated, regenerated. The difference of luminal dilatation of canal of Hering and microvilli decrease in the lumen was observed in chronic liver diseases (p<0.05).
Conclusions: In electron microscopy, immunohistochemistry and PCR in chronic liver disease, canal of Hering was degenerated, which suggests the disturbance of bile flow. This suggests morphologic change may be related to hepatobiliary functional disturbances.

PP-273

Reinvestigation on the efficacy of oxytocin in treating upper gastrointestinal hemorrhage due to liver cirrhosis with portal hypertension

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Objective: To investigate the effect on upper gastrointestinal hemorrhea patients with portal hypertension due to liver cirrhosis treating by oxytocin again.
Methods: Upper gastrointestinal hemorrhea being in hospital were selected in random way to treating group (36 cases) or confronting group(36 cases).In the same basis treatment.200^{U}of oxytocin which was solubilized in 5%GS 500ml were lasting iv gtt as speed of 0.4 to 0.8^{u} per minute in patients of treating group and the speed of drops was changed according the speed of hemorrhage, it also lasted 48-72h after the bleeding was off, 0.4mg of Octreotide which was also solubilized in 5%GS 500ml were lasting iv gtt as speed of 0.5μg per minute in patients of confronting group in the same way.
Results: There were 91.7% effective rate in treating group including hemorrhage stanched in 33 cases and didn't in 3 cases, 91.7% effective rate in confronting group, including hemorrhage stanched in 33 cases and didn't in 3 cases. There were no significant difference between two groups (P>0.05) by the data had been satistically analyzed. There were side effects: 26 cases in treating group and 28 cases in confronting group. There were no significant difference between two groups(P>0.05).The two groups have no serious side effects.
Conclusion: In treating upper gastrointestinal hemorrhea patients with portal hypertension due to liver cirrhosis,the effective of using oxytocin was similar to using Octreotide.

PP-274

Brain water assessed by diffusion weighted imaging correlates with blood ammonia during induced hyperammonaemia in patients with cirrhosis.

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We have previously found that hyperammonaemia induced by oral amino acid challenge in cirrhotic patients is associated with EEG slowing and psychometric abnormalities. The relationship between ammonia level and brain oedema is unclear and thus the purpose of this study was to quantify brain oedema and blood ammonia levels before and after oral amino acid challenge (18g each of the three amino acids glycine, serine and threonine) in eight patients with cirrhosis being assessed for liver transplantation.
Diffusion weighted tensor images (DTI) and T1 anatomical images were obtained for all subjects using a Philips 3 Tesla MR system. Following eddy current correction DTI data were processed to create Mean Diffusivity (MD) maps using a standard algorithm. The anatomical scans were registered to the MD data and segmented to create normal appearing white matter masks. The masks were applied to the MD scans on a slice by slice basis and the average MD was calculated over all slices for every subject.
During the course of the challenge, blood ammonia concentrations increased by 73 $\pm$ 56umol/l (Mean $\pm$ SD) over all subjects. The mean difference in MD of white matter between scans was significantly correlated with increasing NH_3 levels with a Pearson correlation coefficient of 0.7 ($p < 0.04$).
The present approach offers the possibility of directly testing the effect of various therapies to curtail hyperammonaemia or retard the development of cerebral oedema in man in order to minimize the development of overt hepatic encephalopathy.

PP-275

Clinical analysis of pleural effusion occurred in patients with liver cirrhosis

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Ascites and spontaneous bacterial peritonitis (SBP) are well-known complications of cirrhosis, but hepatic hydrothorax (HH) and spontaneous bacterial empyema (SBE) have rarely been reported in Korea. The aims of this study were to determine the incidence of pleural effusion that occurred in cirrhotic patients, and to analyze the clinical characteristics of HH and SBE. We performed a retrospective study through reviewing the medical records of the patients accompanied by pleural effusion among 1,724 cirrhotic patients. Pleural effusion occurred in 6.0% of cirrhotic patients. the most common cause of pleural effusion was parapneumonic effusion (50%), which was followed by HH (43%). The SBE occurred in 30.4% of the patients with HH who received thoracentesis. All cirrhotic patients with HH had ascites, but only part of the patients with SBE had SBP. All seven patients with SBE had decompensated cirrhosis, and three of these patients died of hepatic failure, sepsis and variceal bleeding during admission. HH and SBE are not rare complications of cirrhosis. The cirrhotic patients with SBE are characterized by the markedly decreased hapatic reserve and high mortality. Therefore, for cirrhotic patients with pleural effusion and suspected infection, active thoracentesis is recommended to explore the cause of effusion. Then, appropriate treatments according to causes of effusion are required.

PP-276

Clinical value of the Acti-Test in the estimation of activity of chronic viral hepatitis B/C.

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Non-invasive diagnostics of degree of activity of necro-inflammatory process in hepatic tissue by Acti Test is a new and prospective direction of diagnostics of chronic viral hepatitis. Acti Test includes 6 biochemical parameters combined in discriminant function: ALT, alpha 2-macroglobulin, gaptoglobin, apolipoprotein, g-glutamiltranspeptidase, the general bilirubin.

Estimation of sensitivity (Se) and specificity (Sp) for Acti Test at patients with chronic viral hepatitis B/C at different degree of necro-inflammatory process in hepatic tissue (A0-A3) at the data of biopsy was made. We examined 50 pts with average age 35±10 years. Thirty-three men and seventeen women with BMI= 23.5±4.2 kg\m2 were hospitalized in Vasilenko' Clinic due to chronic hepatitis – 84% (HCVRNA+) and 16% (HBVDNA+).

Acti Test and needle liver biopsy after Mengini were performed at the same day. The results of Acti Test were compared with the data of semiquantitative degree of activity of necro-inflammatory change using Metavir score.

The results of morphological study of liver tissue show that HAI was 6.4 ±1.9 points. Sensitivity (Se) and specificity (Sp) of Acti Test were 68% and 75% for A0, correspondingly; Se - 83%, Sp – 86% for A1; Se - 87%, Sp – 86% for A2; Se – 98%, Sp – 97% for A3.

Acti Test can make a noninvasive estimation of necro-inflammatory changes to liver tissue, and its results are comparable to data of needle liver biopsy. Thus Acti Test can be used as an alternative method of diagnostics at presence of contra-indications to needle liver biopsy of liver at patients with viral hepatitis B/C.

PP-277

CD4-CD8- T cells contribute to the persistence of MHV-3 induced chronic viral hepatitis

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To study the contribution of T cell subsets to the pathogenesis in MHV-3 induced chronic viral hepatitis in C3H/Hej mouse. C3H/HeJ mice received 10pfu of MHV-3 intraperitoneally 63% of MHV-3 infected C3H/HeJ mice developed a chronic course of virus infection characterized by persistence of lymphocyte infiltration and macrophage activation until 40 days post infection of the observed time. The mice underwent fluctuated serum ALT、AST levels and decreased TP、ALB level. The $CD3^+CD4^-CD8^-$ (DNT cells) and $CD4^+CD25^+$T cell ratios raised significantly since 2 days post MHV-3 infection in C3H/Hej mice. DNT cells showed significant and specific cytotoxic effects on $CD8^+$ T cells with a cytotoxic rate of 97.00％±0.01％, while $CD4^+CD25^+$T cells only had slight cytotoxic effects on it with a cytotoxic rate of 51.27%±0.01%. Whereas no effect was observed on hypatocytes with MHV-3 infection or $CD8^+$ T cells and hypatocytes from MCMV infection. The cell surface marker of the DNT cells is αβCD4-CD8-CD25-CD28-CD30-CD44+.+-TCR+ There were minimal amount of infected DNT cells expressing IL-2, IFN-γ one has shown the expression of IL-4 or IL-10.

Conclusions: We first report that an unique group of DNT cells with minor production of IL2, rather than $CD4^+CD25^+$T cells, have more profound immune modulatory effect on $CD8^+$T cells in MHV-3 induced chronic viral hepatitis in C3H/Hej mice, suggesting their contribution to viral persistence. The underlying mechanism are still under investigation.

PP-278

Impact of replenishing-invigorating-clearing-purging method of traditional Chinese medicine on acute hepatitis due to severe acute pancreatitis in early stage

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Objective: To evaluate therapeutic effects of replenishing-invigorating-clearing- purging (RICP) method of traditional Chinese medicine (TCM) on acute hepatitis (AH) due to severe acute pancreatitis (SAP) in early versus advanced stage.

Methods: From January 2003 to June 2004, Fifty-eight patients with AH due to SAP admitted to our hospital were received RICP therapy starting either within 72 h (early-treated group, n = 41) or after 72 h (late-treated group, n = 17) from onset of disease. The two groups had similar baselines at the beginning of study and underwent similar other medical management throughout.

Results: The duration of AH (4.9 ± 7.5 d *vs.* 12.4 ± 18.0 d, $P < 0.05$), enteroparalysis (2.8 ± 2.4 d *vs.* 3.9 ± 3.2 d, $P < 0.05$), acute respiratory distress syndrome (3.0 ± 3.3 d *vs.* 8.9 ± 8.8 d, $P < 0.05$) and acute renal insufficiency (6.0 ± 7.7 d *vs.* 26.8 ± 25.2 d, $P < 0.05$) were shorter in the early-treated group than those in the late-treated group. The early-treated group had lower infection rate (24.4% *vs.* 52.9%, $P < 0.05$), operation rate (14.4% *vs.* 47.1%, $P < 0.05$) and mortality (24.4% *vs.* 41.2%, $P = 0.01$). Moreover, the early-treated group had shorter hospital stay (31.3 d *vs.* 59.4 d, $P = 0.01$).

Conclusions: Early RICP of TCM therapy results in a better clinical outcome in AH due to SAP.

PP-279

The treatment of chronic hepatitis C: role of ICAM-1 and response to interferon.

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Background: The molecules of intercellular adhesion (ICAM-1) play a fundamental role during the hepatic inflammation.

Objectives: The aim of this study is to consider the serum variations of ICAM-1 (s ICAM-1) in patients with chronic hepatitis C (CH-C) with persisting normal ALT levels in comparison with patients with CH-C ad high ALT levels during the therapy with recombinant α 2a interferon (IFN α-2 a). The immunohistochemistry localization of ICAM-1 has been performed also on samples of hepatic tissues of both groups.

Material and Methods: 60 subjects have been divided in three groups:
Group A:19 CH-C with regular ALT levels.
Group B:21 CH-C with elevated ALT levels.
Group C:20 verification group.
The first two groups have been treated with IFN α 2 a with a dose of 6 MU thrice weekly for three months and have continued with 3 MU thrice weekly for another three months.

Results: In groups A and B basic serum ICAM-1 values were significantly higher than those of group C (<0.0001). The average basic sICAM-1 value in group A (525.0 ng/ml) resulted to be lower than group B (561.0 ng/ml), but the difference was insignificant. There is a trend between the elevated ICAM-1 levels and the increase of the histological severity (χ 2mh 8.8, $p<0.003$).

After the treatment the sICAM-1 values demonstrated a clear diminution in both groups, only among the responders patients. The immunohistochemistry localization did not show colouring for ICAM-1 in samples of normal kidneys, but was evident in both patient groups with CH-C.

PP-280

Evaluation of hepatobiliary function in patients with gallstones: comparative study in patients with cirrhosis and non-cirrhosis

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Background and Aims: Impaired gallbladder function has been suggested as a factor to increase prevalence of gallstones in liver cirrhosis. Furthermore, the patients with poor liver function is likely to have gallstones. We compared the hepatobiliary function between cirrhosis and non-cirrhosis patients with gallstones by cholescintigraphy.

Methods: GB stone disease occurred in 19.5% (559 of 2,864) of patients with liver cirrhosis from 2004 to 2007 in our hospital. 22 cirrhosis and 68 non-cirrhosis patients all of whom had gallstones were enrolled. The cirrhosis group consisted of 14 of Child A, 5 of Child B and 3 of Child C. Gallstone number, size and gallbladder wall thickness were checked by abdominal ultrasonography. Quantitive hepatobiliary scintigraphy by TC^{99}m DISIDA was performed. Serial static abdominal images were acquired in 10 min, 30 min, 60 min. The GB ejection fraction (GBEF) was estimated 30 min after fatty meal diet. Hepatic

clearance and small bowel appearing time (SBAT) were additionally obtained.

Results: There were no significant differences in age, sex, body mass index, triglyceride and stone number. GBEF (67.2% vs 61.1%), Hepatic uptake 60 min (151.1 vs 143.6) and SBAT (36.4 vs 41.2) were also not different in the group of cirrhosis and non-cirrhosis. Hepatic uptake 10 min (354.2 vs 510.5) ($p<0.05$) and Hepatic clearance (49.1 vs 68.7) ($p<0.01$) were decreased significantly in the group of cirrhosis compared to non-cirrhosis.

Conclusions: There seems to be no difference of gallbladder function between cirrhosis and non-cirrhosis patients with gallstone. Hepatic uptake and clearance were decreased in cirrhosis patients. Improvement of liver function may contribute to prevent the formation of gallstone.

PP-281

Experimental hepatitis A virus (HAV) infection in Cynomolgus monkeys: findings of liver pathology and extrahepatic HAV replication.

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Non-human primates are an invaluable tool for studies of biology and pathogenesis of hepatitis A virus (HAV), since HAV replication in cell cultures is remarkably slow, and nearly always non-cytopathic. Experimental studies of HAV infection have been carried out in several non-human primates, including both Old World and New World monkeys. Cynomolgus are readily available and easy to handle, however, until recently it is still unknown its susceptibility to HAV. In this study we evaluate the susceptibility of this specie to Hepatitis A virus (HAV) infection. Seven adult animals were inoculated by intravenous route with 1ml of a HAV-containing fecal suspension, with a viral titer of $3x10^5$ copies/µl. After 14th days post-inoculation (dpi), each animal was killed weekly until 59th dpi. Serum, saliva, feces, liver, salivary gland and tonsil samples were collected and stored in liquid nitrogen until analysis. The successful of HAV infection was confirmed by alanine aminotransferase (ALT) elevations levels at 19th dpi and by appearance of anti-HAV IgM and total antibodies in serum at 7th dpi. In addition, HAV-RNA was detected by RT-nested-PCR in serum, saliva, liver, salivary gland and tonsils samples. Besides, cytoplasmatic evidence of HAV antigen was observed in hepatocytes by indirect immunofluorescence. To investigate replication sites, intermediate replicative HAV-RNA was detected in liver and salivary gland samples. In general, necro-inflammatory liver lesions were detected at 14th dpi. In summary our results showed that cynomolgus are susceptible to human HAV and could be used as animal model for pathogenesis and vaccine studies of hepatitis A.

PP-282

Clinical analysis of primary biliary cirrhosis: a report of 9 cases

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Objective: Clinical features of primary biliary cirrhrosis (PBC) were reviewed in order to improve its diagnosis.

Method: The general conditions, clinical manifestations, biochemicaland immunological changes were assessed in 9 patients.

Result: Nine cases were all females, the mean age at definite diagnosis was 52 years. Jaundice was the most frequent symptoms, abdominal distension, pruritus were the second and third, respectively. All patients were complicated by spleen enlargement. ALP, γGT were 3 times more elevated respectively. ALT ,AST and bilirubin levels were mildly or moderately elevated respectively , IgM level was also elevated among 66.7% of patients , 88.9 % of patients, 66.7% of patients had positive ANA, positive AMA-M2 respectively.

Conclusions: The elevatedlevel of AL P ,γGT and IgM and ANA,AMA positive may be crucial to diagnosis of PBC .

PP-283

Chronic active EBV infection - case report

Hong Zhao[1], Jun Li[1], Wang Yan[1], Wang Gui-qiang[1]
[1] *Peking university first hospital*

Epstein Barr virus induces various diseases such as infectious mononucleosis, chronic active EBV infection, EBV associated lymphohistiocytosis, and malignancies. CAEBV is rare but relatively frequent in Asia, especially in Japan. It is characterized by persistent or recurrent infectious mononucleosis like symptoms, marked elevated VCA IgG and EA IgG, and detection of EBV DNA or related antigens in affected tissue, and the absence of evidence of prior immunological abnormality. We report here a previously healthy man who fulfilled the criteria of CAEBV. To our knowledge, this is the first case report in mainland China. A 33 year old man was admitted with 1 year history of fever of unknown original, recurrent skin lesion, slowly progressive weakness of both lower limbs and exacerbated for 1 month. There were non pitting edematous erythema interspersed among eyelid, lips, and submaxillary. Denseness dark papula covered with furfuraceous scale were seen in the patient's face, body and limbs. His inguinal lymph nodes were swollen. Splenomegaly was also found. Laboratory test shown mild lesion of liver (ALT 93 IU/L, AST 20IU/L, ALP 194IU/L, GGT 223IU/L, TBIL 26.5umol/L, LDH 322IU/L, CK 12 IU/L), normal leukocyte count and ESR, RF, CRP. Serum test shown marked increased of anti EBV antibodies (EBV IgM, EA IgG and NA IgG were all positive, VCA IgG 1:20480). EBV DNA in the serum and PBMC by the quantitative real time PCR method were 2100 and 7300000 copies/ml. We did in suit hybridization using his liver and skin biopsy samples and obtained positive result. We prescribed interferon 3MIU three times a week. In summary, CAEBV is a specially type of EBV infection. Careful investigation of hidden CAEBV is very important.

PP-284

Probiotics improve high-fat diet induced steatosis through modulation of hepatic NKT cells

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[1] *Shanghai Jiao Tong University School of Medicine,* [2] *Shanghai Institute of Digestive Disease,* [3] *Johns Hopkins University School of Medicine*

Dietary factors and intestinal bacteria play an important role in rapid increase of the incident of obesity and its associated conditions, such as fatty liver disease and insulin-resistance. Our previous study showed that probiotics, live microbial food supplements, improved steatohepatitis and insulin resistance in *ob/ob* mice. In the current study, we evaluated the effect of probiotics on high fat diet induced obesity, fatty liver and insulin resistance. High fat diet induced a hepatic NKT cell depletion that preceded the formation of insulin resistance and hepatic steatosis. Oral probiotic treatment (VSL#3) significantly improved high fat diet induced hepatic NKT cell depletion, insulin resistance, hepatic steatosis. Adoptive transfer of NKT cells also improved insulin resistance and steatosis. The effects of probiotics on high fat induced insulin resistance and steatosis were NKT cells dependant. In addition, high fat diet also increased the expression of the pro-inflammatory cytokine, tumor necrosis factor-alpha, that activated IKKbeta and reduced the sensitivity of insulin signaling, which were all reversed by either probiotic treatment or adoptive transfer NKT cells. In conclusion, probiotics improve high fat diet induced steatosis and insulin resistance. These effects of probiotic are likely due to modulation of hepatic NKT cells to inhibit inflammatory signaling.

PP-285

Microtubules of the axon in hepatic nerve terminals mediate neurotransmitters

Hyun Joo Song[1], Kyu Won Chung[1], Su Jung Baik[1], Yoon Ju Na[1], Hae Sun Jung[1], Tae Hun Kim [1], Kwon Yoo[1], Il-Hwan Moon [1]
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Backgroud and aims: Stimulation of hepatic nerve increases hepatic metabolism and hemodynamics, reflecting the release of the neurotransmitters from the stimulated hepatic nerve terminals. Precise mechanism of nerve terminals is still unknown. We investigated ultrastructual characteristics of hepatic nerve terminals in chronic liver disease.

Methods: From September 2005 to October 2006, we investigated 52 patients with chronic liver disease (29 males: 23 females), who underwent ultrasonography-guided liver needle biopsy: 12 chronic hepatitis B, 13 chronic hepatitis C, 17 liver cirrhosis, 5 hepatocellular carcinoma, and 5 miscellaneous. Epon embedded specimens were examined by transmission electron microscopy to observe the innervated nerve terminals using Hitachi H7650 electron microscope.

Results: Hepatic nerves were unmyelinated nerve fibers, which composed of several axons. Each axons contained several organelles such as mitochondria, vacuoles, microfilaments, lysosomes, numerous microtubules, and so on. Varicosities of nerve fibers in the connective tissue of portal area were also observed. Contact types of nerve terminals observed 12 with hepatocytes, 13 with hepatic venules, 5 with hepatic arterioles, 5 with bile ductules, and 60 within other connective tissues, respectively. Going near nerve terminals at synaptic sites, microtubules of the axon were typically along with long axis of nerve fibers. Other organelles were not observed there.

Conclusions: These results suggest that microtubules of the nerve

axons may play a main role in neurotransmission in hepatic nerve fibers. Further study is needed to clarify it.

PP-286

Evaluation of liver diseases in Iranian patients with primary antibody deficiencies

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Background: Patients with primary antibody deficiency can complicate with liver disease in several ways, which could be lead to a life-threatening complication in this group of patients. Method: Sixty-two patients with primary antibody deficiencies were followed-up and signs and symptoms of liver disease were recorded. All patients were screened for hepatitis C virus and those patients with any sign of liver disease or gastrointestinal complaints were tested for Cryptosporidium parvum.

Results: Clinical evidences of liver disease were documented in 23 patients (37%). Eight patients (13%) had clinical, radiological and laboratory criteria of chronic liver disease. Only one patient was HCV-RNA positive; he had stigmata of chronic liver disease and pathologic evidence of chronic active hepatitis with cirrhosis. One patient with hyper-IgM syndrome was found to be positive for Cryptosporidium parvum. In liver biopsy of patients with liver involvement, one had histological findings related to sclerosing cholangitis, and five had mild to moderate chronic active hepatitis with unknown reason.

Conclusion: Hepatobiliary disease is a frequent complication in primary antibody deficiencies. Chronic active hepatitis is the most common pathologic feature of liver injury in Iranian patients with primary antibody deficiencies

Poster Session – Molecular and Cellular Biology

PP-287

Stable cell line for secretion of replication-defective hepatitis B virus vector expressing blasticidin resistant gene

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Aims: To construct a stable cell line with permanent secretion of recombinant hepatitis B virus （HBV） vector, which express blasticidin resistant gene.

Methods: Replication-defective HBV vector which express blasticidin resistance gene was constructed. The original genes (Core、Polymerse、pre-S1、pre-S2、S and X) of HBV were deleted by altering the start codon or adding stop codon, then the blasticidin resistance gene was inserted in the S region. G418-resistant replication-defective HBV helper plasmid with deleted packaging signal was constructed. HepG2 cells were cotransfected with both the plasmids stated above. Cell clone selection were done by the addition of both blasticidin and G418.

Results: Over 200 cell clones were formed and 72 were picked and expanded. HBV DNA in the supernatant was tested by dot blot hybridization with isotope labeled probe. After the best 9 clones were maintained for 15 generations, relax circular HBV DNA formation were proved by southern blot with isotope labeled probe. The quantities HBV DNA of the three best cell lines (HBV-Bsd25, HBV-Bsd7, HBV-Bsd27) were $4.1*10^6$, $3.6*10^6$ and $1.2*10^6$ copies/mL respectively. No wild type HBV was detected. Cesium chloride density gradient analysis was done with the concentrated supernatant HBV virion, and enveloped recombinant HBV were proved by southern blot and western blot.

Conclusions: The recombinant HBV secretion stable cell line will contribute to the ongoing selection of HBV infectable cell line, and give impressions for the use HBV vector gene therapy for its overcoming the obstacle of large quantity preparation.

PP-288

FUP1 is a nuclear protein with a bipartite nuclear localization signal

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Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. In general, viral hepatitis and enviromental/chemical agents including hepatitis B viruses, hepatitis C viruses and ethanol intoxication are known to be strongly associated with HCC. Amplification of some growth factors and oncogenes has also been implicated in hepatocarcinogenesis.

FUP1 is a newly identified BTB protein family member. In previous study, FUP1 displayed marked expression difference between human HCC sample and normal liver control. It suggests FUP1 is a candidate oncogene in liver cancer.

In the present study, FUP1 was found to localize in the nucleus of various cell lines. Three putative motifs of nuclear localization signals (NLS) were revealed in a stretch of 20 amino acids between residue 56 and 75. Complete deletion of these 20 amino acids abolished the nuclear localization characteristic of the corresponding mutant protein. Ala scan mutation of basic residues defines that it is a bipartite NLS because either mutation of the first (motif A) or the third (motif C) motif could abolish the nuclear localization characteristic. In conclusion, FUP1 is a nuclear protein with a bipartite NLS. NLS-deficient mutants led to hepatoma cell apoptosis, suggesting that FUP1 is essential for maintaining hepatoma cell survival and is a good target of treating HCC.

Reference:

1. Pan W, Zhang Q, Xi QS, et al (2001) FUP1, a gene associated with hepatocellular carcinoma, stimulates NIH3T3 cell proliferation and tumor formation in nude mice. *BBRC* **286**: 1033-1038.
2. Robbins J, Dilworth SM, Laskey RA, et al (1991) Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. *Cell* **64**:615-623.

PP-289

Epicatechin Abolished TDCA-induced Apoptosis in Huh7 Cell by Inhibiting Bax, p38 MAPK and ROS Production

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[1] *Baylor College of Medicine*

Background and aims: Apoptosis induced by oxidative stress plays an important role in the pathogenesis of various liver diseases. Epidemiological studies demonstrated that dietary flavonoids including epicatechin might reduce the risk of chronic diseases. The aim of this study was to investigate the molecular mechanisms involved in anti-apoptotic effects of epicathechin in liver cells.

Methods: Human hepatoma cell line (Huh7) was treated with 400υM taurodeoxycholic acid (TDCA) for 48 hours to induce apoptosis. Intracellular generation of reactive oxygen species (ROS) was detected with DCFH-DA assay. Caspase-3/7 activity was analyzed with EnzoLyte Homogeneous AMC kit. Cell proliferation was measured by MTT assay. The expression of Bax, Phospho-p38 MAPK and the levels of cytochrome C were assessed by Western- blot analysis.

Results: TDCA-dependent intracellular ROS production was 2-fold higher as compared to untreated cells, consequently resulting in 50 % reduction of cell viability. Interestingly, pretreatment of cells with epicatechin resulted in a dose-dependent inhibition of TDCA-induced ROS generation and reduced cell apoptosis by four-fold as compared to TDCA treatment alone. In addition epicatechin reduced Bax expression with consequential inhibition of cytochrome C release from mitochondria, inhibition of caspase 3/7 activation and p38 MAPK phosphorylation.

Conclusion: Epicatechin protects Huh7 cells from oxidative stress and mitochondria-induced apoptosis. The molecular mechanisms of anti-apoptotic effects of epicatechin were associated with inhibition of p38 MAPK phosphorylation and Bax expression, and reduction of ROS production. These findings implicate epicathechin might have potential as protective agent against a variety of oxidative stress-mediated liver conditions.

PP-290

NS3 protein of hepatitis C virus regulates cyclooxygenase-2 expression through multiple signaling pathways

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Hepatitis C virus (HCV) causes chronic hepatitis, which often results in the development of liver cirrhosis and hepatocellular carcinoma (HCC) worldwide. In this study, we demonstrated that the non-structural protein NS3 of HCV enhances cyclooxygenase-2 (COX-2) gene promoter activity, COX-2 mRNA expression, COX-2 protein production, and prostaglandin E2 (PGE2) release in HepG2 cells in a concentration-dependent fashion. We also showed that transcription factor NF-κB is required for the activation of COX-2 regulated by NS3. In addition, multiple signaling pathways are involved cooperatively in the expression of COX-2 activated by the viral protein in a calcium-independent manner, which requires signaling components including JNK, ERK, and PKD2. A thorough investigation of mechanism involved in the activation of COX-2 regulated by HCV would provide insights into our understanding the processes of liver inflammatory response and hepatocellular carcinoma development caused by the viral infection and also into the development of novel therapeutics against HCV infection.

PP-291

Inhibitive effects of Somatostatin on LPS-stimulated rat Kupffer cells

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[1] *Huazhong University of Science and Technology*

To investigate the effects of somatostatin (SST) on expression of nitric oxide (NO), TNFα and 5-lipoxygenase (5-LO) in primary cultured rat Kupffer cells induced by LPS. Kupffer cells isolated from Sprague-Dawley rats were cultured in the presence of LPS alone or with SST for 12, 24 and 48h, and the production of TNFα were assessed in culture supernatants by ELISA and that of NO by nitrate reducase method. 5-LO mRNA expression was assessed by semiquantitative RT-PCR. Vehicle-stimulated Kupffer cells produced a basal amount of NO, TNFα and expressed 5-LO mRNA. Kupffer cells stimulated by LPS secreted significantly increased amounts of NO and TNFα ($P<0.05$). The secretions of NO and TNFα induced by LPS were inhibited by SST. 5-LO mRNA expression was significantly increased by LPS stimulation ($P<0.05$). Down-regulation of LPS-induced mRNA expression of the 5-LO was also observed in the presence of SST （$P<0.05$）, however 5-LO mRNA expression was still higher than that of control group at 12 and 24h ($P<0.05$), but had no statistically

significant difference compared with control group at 48h ($P > 0.05$). The results indicated that SST modulated NO, TNFα production and 5-LO mRNA expression in LPS-stlimulated Kupffer cells.

PP-292

Induced expressing of PD-1 in Jurkat cell by hepatoma cell and function studying

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Objective: To investigate the effect of hepatoma cells - HepG2 and HepG2 2.2.15 in inducing PD-1(Programmed cell death-1) expression on Jurkat cells, and the function of PD-1 in Jurkat cells.

Methods: HepG2 or HepG2 2.2.15 were co-cultured with Jurkat cells, then stained with PD-1 antibody, PD-1 expression was detected by flow cytometry (FCM), Cytokines in culture supernatant of bloking groups and control groups were measured by enzyme-labeled immunosorbent assay (ELISA) and compared, Cytotoxic action of T cells in bloking groups and controls were measured by methyl thiazolyl tetrazolium (MTT) and compared.

Results: The PD-1 expression on Jurkat cells was induced by Hepatoma cells,the expression rate were 16.17% (by HepG2) and 17.43% (by HepG2 2.2.15) ；the amounts of cytokines in culture supernatant of bloking groups were more(IL-2, 202.9±53.0pg/ml，INF-γ, 88.6±4.6pg/ml，IL-10, 63.7±13.4pg/ml)than that of controls (102.9±53pg/ml，39.3±4.2pg/ml and 34.6±13.7pg/ml，$p<0.05$)；the cytontoxic action—OD value (0.29±0.06) of bloking groups were significantly more than that of controls(0.19±0.09，$p<0.05$) 。

Conclusion: The PD-1 expression on Jurkat cells was induced by Hepatoma cells, and cytokines and cytontoxic action were elevated after blocking PD-1/PDL-1.

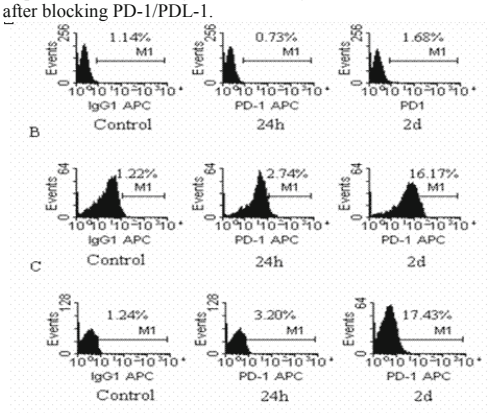

Fig 1.Induced expression of PD-1 on Jurkat cells by hepatoma cells

A：blank group,Jurkat cells

B：Jurkat cells co-cultured with HepG2 cells

C：Jurkat cells co-cultured with HepG2 2.215 cells

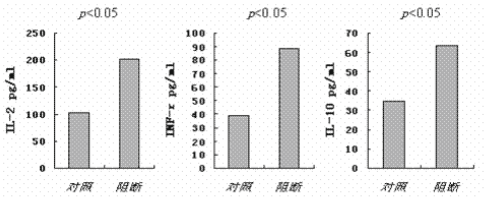

Fig 2.Effect of PD-1/PDL-1 blocking on the cytokines'secretion of Jurkat cells

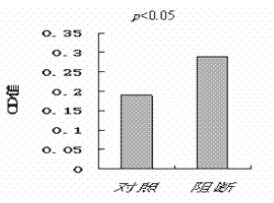

Fig 3.Effect of PD-1/PDL-1 blocking on the cytotoxic action of Jurkat cells

PP-293

The analysis of gene expression profile during H2O2-induced WB-F344 cells malignant transformation

Xuefei Li[1], Yan Li[1], Yinkun Liu[1,2], Kun Guo[1], Dongmei Gao[1]
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Aims: Many evidences suggested hepatic oval cells were the cellular origin of hepatocarcinoma. However, the underlying mechanism remains unclear. Therefore, we investigated the potential molecular mechanisms during malignant transformation of rat oval-like cells induced by H_2O_2.

Methods: WB-F344 cells initiated MNNG were continuously exposed to H_2O_2 to induce malignant transformation which were confirmed by morphology, genetics and soft agar assay. Then, gene expression changes were performed with the functional microarray. The analysis data from microarray was confirmed by Real-time PCR. The proliferous and anti-apoptotic abilities were measured by MTT and cell cycle analysis.

Results: Anchorage independent growth was observed firstly at 5th H_2O_2-induced (WB-5). Transformed cells showed different morphologic characters, such as microvillus and gap junction and displayed heteroploid karyotype at 21th (WB-21). Microarray analysis displayed that the differential expression genes had totally 21 genes which mainly involved the regulation of proliferation, apoptosis, cell adhesion and motility in three groups of cells WB (control), WB-5, WB-21, including *cdk2*, *cdkn1α*, *casp8*, *pten* and *icam1*, *etc*. *pten* was gradually up-regulated in WB, WB-5, WB-21. *cdkn1α* was gradually down-regulated in three groups. MTT and cell cycle analysis displayed the proliferation and anti-apoptotic ability of transformed cells gradually increased, compared control.

Conclusions: These results suggest that the imbalance of proliferation and apoptosis may play a crucial role in hepatocarcinogenesis by H_2O_2-induced. Moreover, *cdkn1α*, *casp8* and *pten* may be important genes in regulating proliferation and apoptosis biological pathways.

PP-294

Contribution of bone marrow mesenchymal stem cells to the maintenance of primary porcine hepatocyte viability and function in vitro

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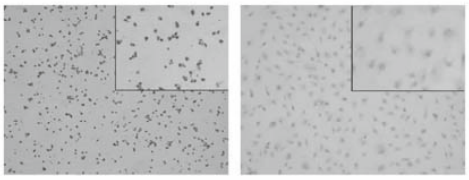
[1] *Department of Hepatobiliary Surgery, Drum Tower Clinical Medical College of Nanjing Medical University,* [2] *Institute of Hepatobiliary Surgery, Nanjing University,* [3] *Department of Hepatobiliary Surgery, Nanjing Drum Tower Hospital*

One of the greatest challenges in the attempt to create functional bioartificial liver designs is the maintenance of porcine hepatocyte differentiated functions in vitro. We compared the morphological and functional changes of hepatocytes cultured with bone marrow derived mesenchymal stem cells (MSCs) at varying ratios. A self-organization of three-dimensional hepatocyte spheroids was encouraged, together with tight junctions among heterotypic cells (Fig. 1 and 2). The results showed a metabolically active, viable cell population in all coculture configurations with few dead cells (Fig. 3A and B). The maximal induction of liver-specific functions was achieved when the proportion of hepatocytes to MSCs was 2 times (Fig. 3C, D, E and 4). Detection of fibronectin rather than laminin confirmed extracellular matrix synthesized by nonparenchymal cells are involved in hepatocyte homeostasis (Fig. 5). Our data also suggested soluble factors secreted by MSCs are the key stimulators in the hepatic functional enhancement (Fig. 6).

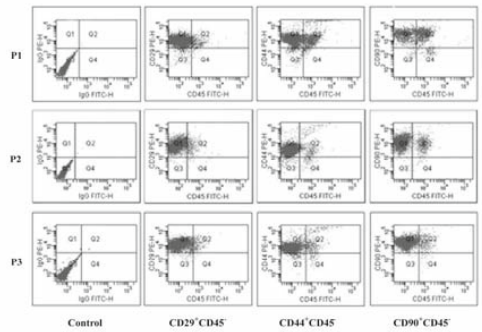
Figure 1A



Hep MSC

Characterization of freshly isolated porcine hepatocytes by immunocytochemistry for albumin

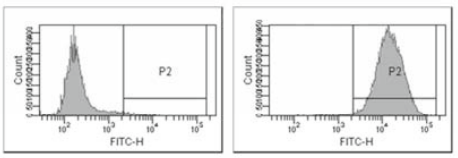
Figure 1B



Control $CD29^{+}CD45^{-}$ $CD44^{+}CD45^{-}$ $CD90^{+}CD45^{-}$

Flow cytometry analysis of CD29, CD44, CD45 and CD90 expression of MSCs

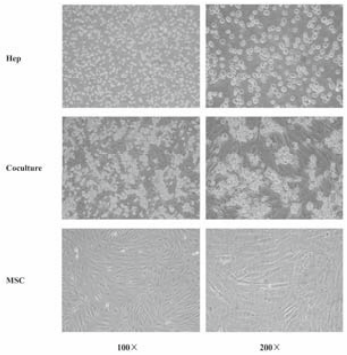
Figure 1C



Control CFSE

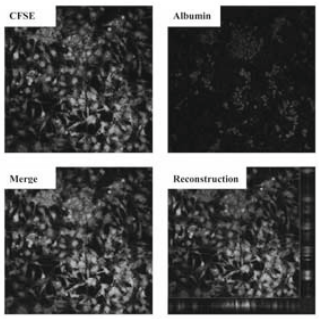
Flow cytometry analysis of CFSE staining for MSCs

Figure 2A



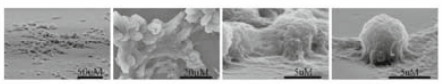
Phase-contrast photographs of hepatocytes cocultured with MSCs

Figure 2B



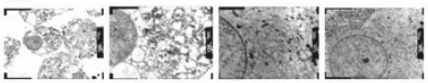
Three-dimensional confocal micrographs reconstructed from sixty sequential scanning images

Figure 2C



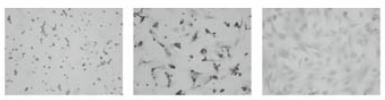
Scanning electron micrographs of hepatocytes cocultured with MSCs

Figure 2D



Transmission electron micrographs of hepatocytes cocultured with MSCs

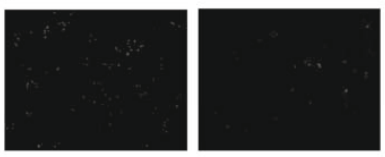
Figure 3A



Hep Coculture MSC

Periodic acid schiff staining of hepatocytes cocultured with MSCs

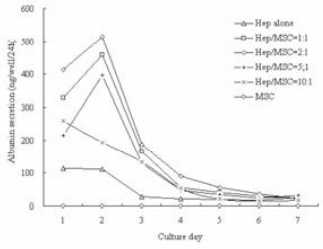
Figure 3B



Hep Coculture

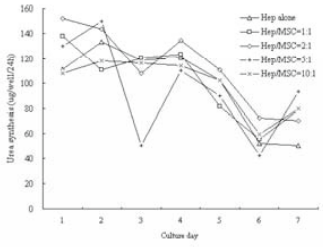
Immunofluorescence staining for nuclei of dead hepatocytes cocultured with MSCs

Figure 3C



Albumin production from hepatocytes during 7 days coculture

Figure 3D



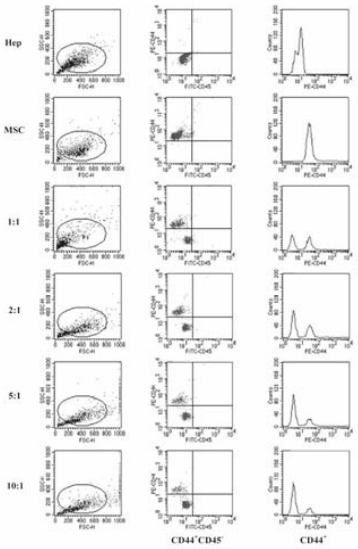
Urea synthesis from hepatocytes during 7 days coculture

Figure 3E

Hep **Coculture** **MSC**

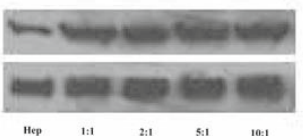
Immunochemistry staining of hepatocytes cocultured with MSCs for intracellular albumin

Figure 4A



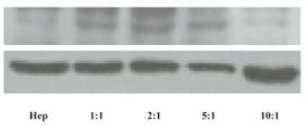
Fluorescence-activated cell sorting of hepatocytes cocultured with MSCs

Figure 4B



Albumin expression of hepatocytes sorted by flow cytometry from coculture with MSCs

Figure 4C

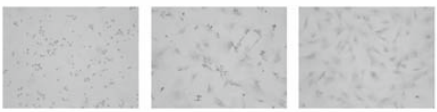


CYP3A1 expression of hepatocytes sorted by flow cytometry from coculture with MSCs

Figure 5A

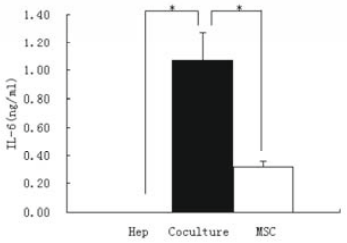
Immunochemistry staining of hepatocytes cocultured with MSCs for extracellular fibronectin

Figure 5B



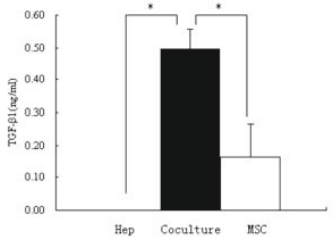
Immunochemistry staining of hepatocytes cocultured with MSCs for extracellular laminin

Figure 6A



Levels of IL-6 in indirect coculture system of hepatocytes and MSCs

Figure 6B



Levels of TGF-β 1 in indirect coculture system of hepatocytes and MSCs

PP-295

Slc25A13 gene mutation is an important reason for Chinese neonatal intrahepatic cholestasis

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Objective: To explore the position of SLC25A13 gene mutations in Chinese infantile intrahepatic cholestasis.

Methods: Blood amino acids were analyzed by using mass chromatography in 115 infants with GGT increasing who were referred to our hospital for further investigations of intrahepatic cholestasis from December 2003 to December 2006. All exons and nearest neighbour sequences of SLC25A13 gene were analysed in 10 patients with at least one of citrulline or methionine double increasing and other 4 with the same amino acids abnormal referred to our hospital after January 2007. SLC25A13 gene two common mutations, Mutation I and mutation III were screened in children without citrulline obviously increased. Sequencing was done in the positive screened patients. All exons and nearest neighbour sequences were analysed in children who had heterozygous mutation.

Results: 8 had SLC25A13 gene mutations in 14 citrullinemia children, including 2 patients with compound heterozygous mutation 851del4/1638ins23, homozygous mutation 851del4/851del4、compound heterozygous mutation 851del4/R184X and homozygous mutation IVS6+1G>A/IVS6+1G>A for each patient, and 3 patients with heterozygous mutation 851del4. Homozygous mutation IVS6+1G>A/IVS6+1G>A was a new mutation first reported in the world . 5 had SLC25A13 gene mutations in other 105 intrahepatic cholestasis patients, including 1 patient with homozygous mutation 851del4/851del4 and 4 patients with heterozygous mutation 851del4. 10 had SLC25A13 gene mutations in all 115 intrahepatic cholestasis patients and the prevalence is 8.7%.

Conclusions: SLC25A13 gene mutation is an important reason in Chinese infantal intrahepatic cholestasis.SLC25A13 gene common mutation pattern in Chinese is different from abroad.

PP-296

Freeze-dried grape powder attenuates mitochondria- and oxidative stress-mediated apoptosis in liver cells

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Apoptosis plays a crucial role in numerous physiological and pathological processes in the liver. The beneficial effects of grape consumption have been attributed to the antioxidant activity of its polyphenols. Although attempts to elucidate the effects of different grape components have been made, the combined physiological effect of grape on cellular level is unclear. To investigate the cytoprotective effects of grapes, mouse and human primary hepatocytes, and human hepatoma cell line were treated with a standardized freeze-dried grape powder (FDGP) obtained from California Table Grape Commission. The titration of FDGP revealed that 300 μg/ml was optimal dose which increase metabolic activity of cells, the phosphorylation of Akt and IκBα, and up-regulate the level of proliferating cell nuclear antigen. To study the molecular mechanisms of anti-apoptotic effects of FDGP, cells were treated with TRAIL (TNF-related apoptosis-inducing ligand), taurodeoxycholic acid (TDCA) or thapsigargin (TG) to induce cell apoptosis through death receptor-, mitochondria- or ER-mediated pathway, respectively. TDCA-induced activation of caspase-3, caspase-7, caspase-9 and Bax was dramatically decreased with co-treatment of FDGP. FDGP reduced levels of Annexin V positive cells by 4-fold. FDGP also restored cellular glutathione content by 71% in cells treated with H_2O_2 to induce oxidative stress. However, FDGP did not inhibit TG induced apoptosis.

In Conclusion: FDGP increased liver cell proliferation and attenuated the oxidative stress- and mitochondria-mediated apoptosis. These data may contribute to our understanding of the mechanisms involved in protective effects of grape in a variety of liver conditions associated with cellular stress.

PP-297

Human umbilical cord blood -derived mesenchymal stem cells for liver cirrhosis

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Background/Aims: Liver cirrhosis is characterized by extensive fibrosis scarring of the liver and not a reversible process in general. Here, we investigated the effect of infusion of human umbilical cord blood (HUCB)-derived MSCs (HMSCs) in liver cirrhosis rats.

Methods: HMSCs were infused to rats by tail vein to which liver cirrhosis was induced by carbon tetrachloride (CCl4) for 8 weeks. The effect of HMSCs on liver cirrhosis was evaluated using hematoxylin and eosin (H&E) staining, and Masson's trichrome (MT) staining. Serums were collected for laboratory tests. Immunohistochemistry,

Results: H&E and MT staining showed that liver fibrosis in rats was more alleviated when HMSCs were infused than those without HMSCs. HMSCs, were labeled with CM-DiI, autologously transplanted into CCl4- injected rats and CM-DiI labeled HMSCs were expressed the hepatocyte-specific markers, human albumin (hAlb) and alpha fetoprotein (hAFP). Additionally, improvements of serum biochemical markers in the infused HMSCs group were observed. In

immunohistochemistry, RT-PCR, and Western blot, fibrosis markers were also decreased.
Conclusions: HMSCs infusion ameliorated biochemical markers and histologic findings of liver in rats with CCl_4-induced liver cirrhosis.

PP-298
Expression of Clotting factors in Endothelial-like Cells generated from Human Monocytes
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The aim of this project was to establish an in-vitro differentiation protocol of human blood monocytes into endothelial-like cells. Programmable cells of monocyte origin (PCMO) were chosen as a starting source material. PCMO were produced as described. Briefly, monocytes were cultured for 6 days in "dedifferentiation medium" containing ß-mercaptoethanol, M-CSF and IL-3. Cells were proliferated under the influence of human IL-3 and M-CSF and termed PCMO. On day six, the dedifferentiation medium was replaced by differentiation medium with different growth factors namely EGF, aFGF and bFGF. Morphological changes of the cells were observed by phase-contrast microscopy during the incubation of the cells in the differentiation medium. The cells swelled and became polygonal shape contained up to four nuclei. Immunohistochemical staining showed the cells stained positive for vWF (von Willebrand Factor). The gene expression analysis showed that the endothelial markers CD 31, CD 105, vWF and ICAM and coagulation markers Factor I, VII, VIII, IX, and X. The cells expressed also CD 14, a marker of monocytes, and Transferin. In comparison, hepatocyte-like cells from Eufets AG treated with HA-Medium consisted of RPMI based medium with HGF and FGF4 showed the same gene expression profile. Injection of cells resulted in vWF positive structure suggesting an endothelial cell differentiation in vivo.
In conclusion: endothelial-like cells express endothelial and coagulation factor markers.

PP-299
The effect of thymosin alpha 1 on expression of signal transduction molecules in peripheral blood lymphocytes by gene chip
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Background: In the present study, we reported the effect of thymosin α1 on expression of signal transduction molecules in peripheral blood lymphocytes by gene chip.
Methods: Human peripheral blood lymphocytes for experimental group stimulated by Tα1 and control group were isolated from three normal blood. After cultured for 48 h by adding T α1, the total RNA were extracted. The fluorescent cDNA probes were then prepared through reverse transcription labeled with cy3-dUTP and cy5-dUTP, for control group and experiment group, respectively. After hybrid, the gene chip was scanned by ScanArray 5000 laser scanner at two wavelength. The acquired scanning image was analyzed by GenePix Pro 3.0 software.
Results: Human peripheral blood lymphocytes stimulated by Tα1，the expression levels of 61 genes changed and 22 of them down-regulated while the other 39 genes up-regulated.
Conclusion: The genes encoding CAML, PI-3K, TSP2, HM74, PTP and PSTP were up-regulated, and the gene encoding LOK down-regulated, it was related to the anti-tumor function of Tα1. The genes encoding NOEY2 and TSP2 were up-regulated, and it was related to the anti-tumor function of Tα1. The genes encoding P84, nerve-endocrine specific protein A and progesterone binding protein were up-regulated, and it was related to the effect of Tα1 on immune-nerve-endocrine system. The genes encoding Cu/Zn SOD were up-regulated, and it was related to anti-consenescence function of Tα1.

PP-300
Inhibition of the PI-3K/AKT Pathway induces MKP-1 expression and up-regulation of MEK1/2 pathway in cholangiocarcinoma cells
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In cholangiocarcinoma (CCA), several signaling pathways are constitutively activated. Among them, the PI3K/AKT signaling pathways are activated through multiple mechanisms and appear to play a major role in CCA cell proliferation and invasion. In this study, the effects of PI3K/AKT inhibitors (worthmannin and LY294002) were tested in cholangiocarcinoma cell lines (RMCCA1, KKU100 and KKUM213). We found that inhibition of PI3K/Akt signaling pathway induced RMCCA1 and KKU213 apoptosis. However, inhibition of PI3K/Akt signaling pathway in KKU100 induced the expression of Mitogen-activated protein kinase (MAPK) phosphatase (MKP)-1 which inhibits cancer cell apoptosis by de-phosphorylation of p38 MAPK. Furthermore, we found that attenuation of p38 MAPK phosphorylation decreased activity of protein phosphatase 2A (PP2A) and up-regulation of the MEK-ERK pathway. Importantly, phosphorylation of Bad at Ser-112 was found to be restored by up-regulation of the MEK1/2 pathway. In summary, the present data indicated a novel MKP-1-mediated survival pathway in cholangiocarcinoma cell received PI3K/AKT inhibitors. These findings provided the interesting data on a potential role for the inhibition of signal transduction molecules in the treatment of cholangiocarcinoma.

PP-301
Increased MARCKS expression associated with NF-kB in cholangiocarcinoma induced by liver fluke (Opisthorchis viverrini)
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Myristoylated alanine-rich C kinase substrate (MARCKS) is the membrane-bound protein that implicates in coordination of membrane–cytoskeletal signaling events, such as cell adhesion, migration, secretion, and spreading. Our previous study demonstrated that MARCKS overexpression was found in Opisthorchis viverrini (OV)-associated cholangiocarcinoma (CCA) in hamster model. The predominant expression was significantly increased in the early stage of carcinogenesis and also in fully developed CCA. At the early stage, MARCKS expression was found significantly in inflammatory area where the NF-kB expression was co-localized suggesting that MARCKS may involve in inflammation-associated carcinogenesis. The co-expression between MARCKS and NF-kB induced by inflammation was also confirmed by incubating Raw 264.7 macrophage cell line with lipopolysaccharide (LPS) and/or crude OV antigens. The data showed that both LPS and crude OV could induce MARCKS and NF-kB protein that were simultaneously increased and reached the highest intensity at 18 hr of incubation. This finding can be concluded that MARCKS and NF-kB was associated with inflammation-related CCA induced by OV. Further study on molecular mechanism of MARCKS and NF-kB that involve cellular function is under investigation. The understanding of specific pathway in cholangiocarcinogenesis can serve for cancer prevention and treatment in the area where the liver fluke is endemic.
This work is supported by KKU-49 grant of Khon Kaen University and Thailand Research Fund through Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0070/2548) to AT and PY.

PP-302
Decreased adiponectin and antioxidant enzymes are associated with necroinflammatory changes and fibrosis in a rat model for non-alcoholic fatty liver disease (NAFLD)
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NAFLD is a chronic liver disease characterized by fatty liver, necroinflammation and fibrosis. We fed a diet containing 30% fish oil to female Sprague-Dawley rats (180-200g), consumed ad libitum for 8 weeks (NAFLD; n = 8). Control animals (CF; n = 8) were fed with isocaloric rat chow. At killing, blood and liver samples were collected for serum alanine aminotransferase (ALT), histology and molecular analysis. Histological sample was evaluated for fatty liver, necrosis and

inflammation. Collagen was estimated based on the degree of Sirius Red staining. RT-PCR was carried out for adiponectin, glutathione peroxidase (GPx), superoxide dismutase (Cu/Zn SOD), catalase (CAT), TGF-β1, procollagen (Pro-col), tissue inhibitors of metalloproteinases (TIMP-1; TIMP-2) matrix metalloproteinase (MMP-2). Zymography was performed to determine the enzyme activity of MMP-2. Electrophoretic mobility shift assay was done for nuclear factor-kappa B (NF-κB) activity.

NAFLD rats had significantly higher serum ALT, fatty liver, necrosis, inflammation and amount of collagen formation. The mRNAs of GPx and CAT were reduced in NAFLD rats but the Cu/Zn SOD level was not altered. Liver adiponectin was also significantly diminished in NAFLD rats. mRNA levels of TGF-β1, Pro-col, TIMP-1, TIMP-2 and MMP-2 were also significantly upregulated in the NAFLD group when compared to CF rats. MMP-2 enzyme and NF-κB activity were also markedly increased in the NAFLD rats. Our study shows that fatty liver and necroinflammation are accompanied by reduction in the antioxidant enzymes (GPx and CAT) and adiponectin. Liver fibrosis, another important feature of NAFLD was accompanied by upregulation of profibrogenic mediators.

PP-303

Heat shock protein 70-kDA (Hsp70) in HepG2 cells exposed to copper and Jackson toxic milk mouse model of Wilson Disease (WD)

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Heat Shock Proteins (HSPs), notably the inducible 70-kDa protein, Hsp70, participate in cytoprotection during cellular stress including oxidant stress. WD is a genetic disorder of hepatic handling of Cu, where hepatocellular damage involves oxidant stress. We identified Hsp70 as a member of the Cu-metalloproteome (J Proteome Res 2004; 3:834).

Aim: to examine the expression of Hsp70 in the human hepatoma line HepG2 exposed to copper and in liver from the Jackson toxic milk mouse (G712D missense mutation in Atp7b gene, called "tx-j" here).

Methods: HepG2 cells were cultured in increasing concentrations of CuCl (0-50-100-150 uM) for 24 and 48 hours; incubation with ZnCl over the same concentration range were used as control. Hsp70 protein and mRNA expression were examined by immunoblotting and semi-quantitative RT-PCR, respectively. The same analyses were performed on the livers of tx-j mice and control mice, 1-6 months-old.

Results: The Hsp70 protein concentration in HepG2 cells incubated in Cu for 24 and 48 hours demonstrated a dose dependent respond to increasing concentrations of Cu. HepG2 cells incubated in Zn up to 48 hours showed no increase in Hsp70 protein expression.There was no up regulation of Hsp70 mRNA expression despite an increase in protein expression. In the tx-j mouse, there was a non-specific expression pattern of Hsp70 protein and mRNA. In contrast, the control mice demonstrated a gradual increase in Hsp70 protein expression, in parallel with developmental age. A concordant pattern of Hsp70 mRNA expression was also noted.

Conclusion: Changes in Hsp70 expression in HepG2 cells exposed to Cu are consistent with a response to oxidant stress. In Wilsonian (tx-j) mice Hsp70 appears to play a limited role in physiological adjustments as a result of impaired hepatic Cu-handling.

PP-304

Anesthetic propofol protects hepatocytes from oxidative stress-induced apoptosis through activation of Extracellular Signal-Regulated Kinases (ERK) pathway

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Propofol protects cells against ischemia/reperfusion (I/R) injury in several organs, but its effect on liver epithelial cells is few reported. Here we investigated the effect of propofol preconditioning on human hepatic L02 cells under hydrogen peroxide (H_2O_2)-induced oxidative stress and attempted to find out whether ERK pathway is involved in this process. Preconditioned or nonpreconditioned human hepatic L02 cells were exposed to H_2O_2 and the changes of apoptosis were evaluated by TUNEL assay, Caspase-3 and PARP cleavage. Activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and MAP Kinase/ERK Kinase 1/2 (MEK1/2) was measured by Western blot analysis. The mRNA expression of *Bcl-2*, *Bcl-x_L*, *Bad,* and *Bax* was quantified by real-time quantitative RT-PCR. Propofol preconditioning reduced the population of apoptotic cells and Caspase-3 and PARP cleavage induced by H_2O_2 in hepatic L02 cells. L02 cells treated with propofol (0.01 – 0.3 mM) alone, led to a dose-dependent activation of ERK and MEK, and such activation was detected within 0.5 h and eventually declined to less than 50% at 4 h. The addition of specific inhibitor PD98059 completely abolished the activation of ERK and aggravated the extent of apoptosis. Moreover, propofol treatment repressed the mRNA expression of pro-apoptotic genes *Bad* and *Bax*, and this repression could be partly reversed by PD98059. These findings demonstrate that propofol protects hepatic L02 cells from H_2O_2-induced apoptosis, partly through activating MEK-ERK pathway and further suppressing *Bad* and *Bax* expression.

PP-305

Association study between MTHFR polymorphism (C677T) and non familial colorectal cancer in Iranian patients

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The enzyme 5, 10-methylene-tetrahydrofolate reductase (MTHFR) is linked to DNA methylation, synthesis and repair. The single nucleotide polymorphism C677T has been found to be associated with decreased enzyme activity and reduced plasma folate, thus it may play a role in the etiology of colorectal neoplasia. Using pyrosequencing, we analyzed the MTHFR genotypes in 118 colorectal cancer patients and 189 normal controls. We also examined whether the association was modified by folate intake. Whereas the CC, CT and TT genotypes of *MTHFR* among the colorectal cancer patients were 51.7%, 28.1% and 20.3%, we could find 47.1% of 677CC versus 27.0% of 677CT and 25.9% of 677TT in the normal controls, respectively. An inverse association between colorectal cancer risk and the TT genotype was determined, with odds ratios (OR; and 95% confidence intervals) for the CC, CT, and TT genotypes of 1.00, 1.12 (0.77-1.07), and 0.96 (0.59-1.68). We observed an increased risk of colon cancer when folate intake was low for participants with wild type genotypes. This association was similar in both sexes, stronger at high levels of folate intake. Our study corroborates previous findings of an inverse association of the MTHFR 677TT genotype with colorectal cancer, especially at high levels of folate.

PP-306

Effects of highly metastatic hepatocellular carcinoma cell surface-binding peptide on the invasion and metastasis of hepatocellular carcinoma

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Objective: To study the effects of highly metastatic hepatocellular carcinoma (HCC) cell surface-binding peptide (AWYPLPP peptide) on the invasion and metastasis of HCC.

Methods: The effects of AWYPLPP peptide on the invasion, migration, proliferation, adhesion, and activity of matrix metalloproteinases (MMPs) of highly metastatic HCC cell line （HCCLM3） were evaluated in vitro. After transplantation of 5 × 106 HCCLM3 cells s.c. for 10 days, 27 mice bearing HCC xenografts were randomized into three groups (n = 9 in each). Synthetic cognate or control peptide was injected into the subcutaneous tissue adjacent to the tumor at a dose of 100 μg/mouse in 200 μl vehicle (PBS) every 2 days. After 30 days of transplantation, all mice were killed and lung metastases were recorded.

Results: Incubation with the AWYPLPP peptide, but not the control peptide, resulted in a concentration-dependent increase in HCCLM3 cell invasion. At any concentration used for the invasion assay, the peptide had no effect on cell adhesion, proliferation, and adhesion. Increased activity of MMP-9 was observed after incubation of HCCLM3 cells with the AWYPLPP peptide, but not with the control peptide. Eight of ninemice in the AWYPLPP peptide group showed lung metastasis. The metastatic ratio of lung metastasis was significantly increased in the AWYPLPP peptide group compared with the PBS group.

Conclusions: AWYPLPP peptide was able to promote in vitro invasion and in vivo lung metastasis of highly metastatic HCC cells. The mechanism of AWYPLPP peptide may be associated with activation of MMP-9.

PP-307

NIS is expressed by hepatic progenitor cell/ ductular reaction in human liver diseases

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Background & Aims: Sodium iodide symporter (NIS) is an plasma membrane glycoprotein that mediats transport of iodide into several extrathyroidal tissues as it does in thyroid follicular cells. The use of NIS as a reporter gene has recently provided a means to image and monitor the activities of tumor cells, immune cells and neuronal stem cells with radioiodide. The aim of our study is to characterize the expression pattern of NIS in a range of liver diseases and investigate the relationship between NIS and hepatic progenitor cells.

Methods: NIS expression was studied by immunohistochemistry on human liver specimens from patients with hepatitis B virus (HBV)-related cirrhosis (n=15), primary biliary cirrhosis (n=9), extrahepatic subobstruction (n =8) and focal nodular hyperplasia (n= 5). Hepatic progenitor cell was studied with cytokeratin 19- (CK19-) and Hepatocyte- (Hep-) stained serial sections.

Results: In normal human liver, NIS expression was present at the plasma membrane of bile epithelial cells. Immunohistochemical analysis revealed marked NIS immunoreactivity in ductular reaction. On serial, immunostained sections, a small number of cells in the ductular reaction were positive for CK19, Hepatocyte, and NIS at the same time. Hepatocytes remained negative in all of the samples examined.

Conclusions: Human progenitor cells exist in ductular reaction. NIS positivity in the progenitor cells/ductular reaction may permit radioiodine to monitor cell activity.

PP-308

Hemochromatosis screening in a population of students of the Brianza Area

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Hereditary hemochromatosis (HH) is a genetic disorder of iron metabolism resulting in excessive iron overload. Our pilot study, promoted by Lions Club "Brianza Host", aims to evaluate the utility of a phenotypic screening in a population of students of the Brianza Area (Northern Italy) and the subsequent research of genetic mutations associated to HH. From 2004 to 2006 we studied 2024 subjects (1115 M and 909 F, mean age 19,8 years) by determining plasma iron and transferrin and calculating the percent of transferrin saturation (TS%). Subjects with TS% >45 were re-contacted in order to repeat biochemical tests and to undergo molecular detection of 18 known genetic mutations in HFE, TFR2 and FPN1 genes.

Among 229 (11%) subjects with TS% >45 (60 F and 169 M), 104 (45%) were controlled (18 F and 86 M) with biochemical and molecular tests. In 49 subjects, mutations in HFE gene were detected: 34 H63D heterozygotes, 3 H63D homozygotes, 5 C282Y heterozygotes, 2 compound heterozygotes C282Y/H63D, 1 C282Y/S65C and 1 H63D/S65C, 3 S65C heterozygotes. TS% >45 was confirmed in 22 out of 49 (45%) subjects with genetic mutations and in 11 out of 55 (20%) with wild type genotype.

In 7 males: 2 C282Y/H63D and 1 C282Y/S65C, 2 C282Y heterozygotes and 2 H63D homozygotes were detectable mild biochemical alterations (TS%= 68, 94, 74, 50, 85, 60, 51 respectively). The compliance of the study was acceptable (45,4%) and we detected a group of young subjects (7/2024=0.34%) with mild biochemical alterations in iron metabolism related to HFE risk genotypes.

PP-309

Increased expression of Integrin β1 subunit negatively regulate cell growth through the induction of CDK inhibitors in hepatocellular cancer cell lines

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Integrins, heterodimers of α and β subunits, are a family of cell surface molecules mediating cell-cell and cell-extracellular matrix interactions. Research in recent years has indicated that malignant cells possessed deletions or decreased expression of most integrin subunits. In our previous works, we found protein and mRNA amounts of integrin α5β1 subunit are much lower in human hepatocellular carcinoma cells than in normal hepatocytes. Stable overexpression of integrin β1 subunit caused S-phase delay and growth inhibition in human hepatocellular cancer cell line SMMC-7721 cells. This might be due to the increased expression of the CDK inhibitors, p21 and p27, after integrins overexpression. Similar results were also found in other liver cancer cell lines such as HepG2, MHCC97-H. We also found solely overexpression of integrin β1 subunit could induce expression of α5 subunit in certain cell line which is required for its growth inhibitory function, though the mechanism remained unknown. And both the JNK pathway and PI3k/PKB pathway were found inhibited after β1 subunit were stably overexpressed, which were proved to be responsible for the accumulation of protein p21 and p27.

PP-310

Improvement of cultural method in vitro for Echinococcus and culture product initial identification

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Objective: To Improve cultural method in vitro for E.m metacestodes , and identify its DNA. Methods After recovery Bel-7404 cells, and reserve its supernatant fluid，metacestode tissue placed in DMEM medium containing supernatant fluid was cultured in an incubator containing 5% CO2 at 37℃ for a period of 27 days. Follow-up parameters were recorded during cultivation included the counting, growth, measurement diameters, and drawing growth curve of secondary vesicles. After the culture finished, the secondary vesicles' hydatid fluid and wall were detected with protein level and DNA identification. Results Echinococcus multilocularis metacestodes could develop in DMEM medium containing Bel-7404 cells supernatant in vitro. The vesicles' diameter was from 0.5 to 5mm. Detection of secondary vesicles' hydatid fluid protein level in vitro were 1.8mg/ml and PCR amplification of vesicles' wall DNA showed the same specific 200bp band as Echinococcus multilocularis. Conclusion 1. Echinococcus multilocularis metacestodes growth in vitro did not rely on Bel-7404 cells, completely. 2. Different culture environment in vitro and in vivo resulted in different protein level. 3. DNA identification confirmed that the secondary vesicles are Echinococcus multilocularis metacestodes.

PP-311

Construction of the miRNA expression plasmids of human fgl2, Fas and TNFRI genes and their effects in vitro

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This study was designed to explore the opportunity of microRNA interference technique in the inhibitory application of human fgl2, human Fas and TNFR1 expression. The eukaryotic expression plasmids of human fgl2, Fas and TNFRI gene were constructed and have been shown successfully to express hfgl2, hFas and hTNFRI protein. miRNA expression plasmid of hfgl2, hFas and hTNFRI named p-hfgl2miRNA, p-hFasmiRNA and p-hTNFRI miRNA complimentary to the sequence responsible for hfgl2, hFas and hTNFRI respectively were also constructed, meanwhile irrelevant miRNA plasmid was used as control. By respectively cotransfection of p-hfgl2miRNA and pcDNA3.1-hfgl2, p-hFasmiRNA and pcDNA3.0-hFas, p-hTNFRI miRNA and pcDNA3.0-hTNFRI expression construct into 293T cells, the inhibition of hfgl2, hFas and hTNFRI expression was analyzed by quatitative real time PCR and western blot. The experiments showed the significant inhibitory effect of p-hfgl2miRNA on hfgl2, p-hFasmiRNA on hFas and p-hTNFRI miRNA on hTNFRI expression at 48h post-transfection both at RNA level and at protein level as well in 293T cell lines with the inhibitory efficiency reached as high as 89.3% for hfgl2, 87.5% for hFas and 80% for hTNFRI, respectively. The study demonstrated that construct of p-hfgl2miRNA, p-hFasmiRNA and p-hTNFRI miRNA successfully interfered their target genes expression in vitro and these provide the foundation for further investigation of these constructs' application in vivo and further more as a therapeutic strategy for a targeting intervention in the disease control to which the gene fgl2, Fas and TNFRI contributed.

This work was supported by No.30571643, No.30672380, No.30700702; 2005CB522901, 2007CB512900

PP-312

Cholesterol metabolism in Cultured Steatotic Hepatocytes

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Aim;To study Association between steatotic hepatocytes and increased risk of cardiovascular disease.
Methods: Steatosis model of hepatocytes was established by adding palmic acid and oleic acid (oleate/palmitate, 2:1 ratio) to the HL-02 cells. The cells were collected at 12h, 24h and 48h. The cells added culture solution without palmic acid served as control. The intracellular triglyceride and total cholesterol were detected by using analyzed kit. The expression of SREBP-2, hydroxymethylglutaryl CoA reductase (HMGCR), low density lipoprotein receptor (LDLR) and cholesterol 7alpha-hydroxylase (CYP7A1) was measured with RT-PCR.
Results: Hepatocyte steatosis was observed at 12 hour, and became more serious at 24h and 48h. The contents of intracellular triglyceride and cholesterol increased, and the expression of SREBP-2 and HMGCR was up-regulated in a time-dependent manner in model group, however LDLR and CYP7A1 were down-regulated. Compared with control group, the content of intracellular triglyceride and cholesterol were higher at all model groups ($P<0.01$ respectively); The expression of SERBP-2 and HMGCR mRNA was up-regulated in steatotic hepatocytes ($P<0.05$ respectively) while the LDLR and CYP7A1 mRNA were down-regulated($P<0.05$).
Conclusion: More synthesis and less elimination of cholesterol in steatotic hepatocytes increase the risk of cardiovascular disease. These show the possible role of NAFLD in the development of cardiovascular disease (CVD).

PP-313
Effect of cytokines on expression of DCN mRNA of HSC-T6
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Objective: To study the regulation effect of cytokines on expression of DCN mRNA.
Methods: To regarding HSC-T6 as the target cell, we study effect of PDGF、IFN、TNF-α and IL-6 on expression of DCN mRNA by technology of RT-PCR.
Results: IFN-γ inhibits cell multiplication and increases expression of DCN mRNA in the concentration of 105 –106u/ml, whereas inhibits decreases expression of DCN mRNA in the concentration of 10^2-10^3u/ml; TNF-α decreases expression of HSC DCN mRNA in the high concentration (50pmol/l); PDGF have obvious regulation effect on DCN mRNA in the condition of PDGF10ng/ml and 10% FSC; IL-6 decreases the level of DCN mRNA of HSC-T6.
Conclusion: IFN-γ and TNF-α have the double –acting regulatory effects on expression of DCN, and it happens in the level of gene transcription and after it; And regulation effect of PDGF on proliferation correlates to expression inhibition.

PP-314
Protective effect of α-Lipoic acid on ischemia-reperfusion injury in rats liver
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Objective: To explore the effect and mechanism of α-Lipoic acid on ischemia-reperfusion injury in rat river. Methods The models of liver partial ischemia-reperfusion were established in male Wistar rats. The rats were randomized into a control group (group A) and an experimental group (group B) and administered either saline or α-Lipoic acid in 15 min before the ischemia. GPT、GSH and GSH-PX were examined at various time after reperfusion (1h, 3h, 6h, and 12h) in serum of the rats with or withoutα-Lipoic acid administered. Expression of NF-κB P65 was evaluated by immunohistochemistry assay and expression of MIP-2mRNA was detected by RT-PCR in ischemic liver. Results Compared with group B, the ALT level and the activity of GSH-PX in serum, the expression of NF-κB P65 and MIP-2mRNA in ischemic liver of the rats in group A were significantly high ($P<0.01$), while GSH in serum was lower ($P<0.01$). Conclusion α-Lipoic acid may protect rats from liver ischemic reperfusion injury through cleaning oxygen free radicals, increasing GSH production, and reducing NF-κB activation and GSH consumption.

PP-315
Experimental Modeling SD Rats Metastatic Hepatocellular Carcinomas Induced by DEN and NMOR
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Objective: To establish DEN and NMOR-induced metastatic hepatocellular carcinomas model in SD rats and investigate proper dose and treatment period.
Methods: One hundred and ten 5-week-old male SD rats were randomly divided into four groups: DEN+NMOR, DEN, NMOR and control group. Group DEN+NMOR(60 Rats) and Group DEN (20 Rats)were given a single i.p. injection of DEN as an initiator of liver carcinogenesis at a dose of 100 mg/kg body weight. And then groups DEN+NMOR, NMOR (20 Rats) were given 100 ppm NMOR in the drinking water for 20 weeks. Control group (10 rats) was only given a single i.p. injection of sodium chloride as the same volume as DEN solution.
Results: By the end of 16 weeks, the HCCs incidences in group DEN+NMOR, DEN, NMOR and control group were 60.0%(6/10), 20.0%(1/5), 0.00%(0/5), 0.00%(0/2). And, by the end of 20 weeks the HCCs incidences in group DEN+NMOR, DEN, NMOR and control group were 87.5%(21/24), 16.7%(1/6), 14.3%(1/7), 0%(0/2), respectively. Lung Metastases incidences in group DEN+NMOR at the end of 16th week and 20th week were 31.3%(2/6), 47.6%(10/21), which were not observed in other groups.
Conclusions: The model on hepatocarcinogenesis induced by DEN and NMOR in SD rat provides an efficient means for rapid induction of metastatic HCC and represents the natural course of metastasis of malignant tumors. A single injection of DEN as an initiator of carcinogenesis at a dose of 100 mg/kg and sequentia 100 ppm NMOR in the drinking water for 20 week probably is an appropriate treatment.

PP-316
The rate of glycogen accumulation in normal and cirrhotic liver of 48-hours fasted rats after oral glucose load
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In patients with liver cirrhosis, carbohydrate metabolism has been known to be disturbed. Since the liver plays the key role in maintenance of the constant blood glucose level, deterioration of glucose absorption and of its deposits in hepatocytes in the glycogen might be of major importance in development of glucose intolerance in patients with liver cirrhosis.
In the present work, dynamics of glycogen accumulation was studied in the normal and cirrhotic rat liver (6 months of CCl_4 inhalation) at 10, 20, 30, 45, 60, 75, 90, and 120 min after administration of 30% glucose (4 g/kg body mass) to 48-hours fasted animals. Material from each animal was studied by three different methods: biochemical, cytofluorimetric and of absorption television cytophotometry.
According to data of different methods, glycogen accumulation in the cirrhotic liver after an oral glucose administration to fasting rats starts after a 20-30-min delay. The intensity of glycogen accumulation in the cirrhotic liver is 26 % lower than in the norm, which is due to a lower rate of glycogen synthesis in the pathologically changed liver. It has been shown that hepatocytes of the portal lobule zone both in the normal and in the cirrhotic liver at all stages after the glucose administration accumulate more glycogen than hepatocytes of the central zone. The glycogen content in hepatocytes of the portal and central lobule zones of the normal liver was higher than that in cells of the corresponding zones of the cirrhotic liver, on average, by 18 and 14 %, respectively.

PP-317
Expression of K18 phosphorylation in apoptosis of hepG2 cells
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Background: Ser33,Ser52 phosphorylation of Keratin 18 have been proved to exert important function in human liver disease and can be a marker of progression of human liver disease.The aim of this article is to study the relationship of phosphorylated keratin 18 (Ser33,Ser52) and apoptosis of human hepatocytes. Method: Apoptosis was induced in HepG2 cells by CDDP. Cells was stained by Annexin V and PI, then analyzed by Flow cytometric to evaluate apoptosis. Protein levels of K18 and Ser33,Ser52 phosphorylation of Keratin 18 in apoptosis cells were analyzed by immunofluorescence and Western Blotting. Results: The apoptosis rate of HepG2 cells increased along with the increase of CDDP concentration. The protein level of Ser-52 phosphorylated keratin 18 increased along with the concentration of CDDP. The protein level of Ser-33 phosphorylated keratin 18 increased in low concentration of CDDP, but decreased evidently in high concentration

of CDDP. Conclusions: Apoptosis could be induced in HepG2 cells by CDDP. Ser33, Ser52 phosphorylation of Keratin 18 have close relationship with apoptosis of human hepatocytes.

PP-318

The significance of BSEP mutations in progressive intrahepatic cholestasis children with Low γ-GT in a tertiary pediatric hospital in Eastern China

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Objective: To clarify the significance of BSEP mutations in progressive intrahepatic cholestasis children with low γ-GT in eastern China.

Methods: From January 2004 to June 2007, 24 children had presented progressive intrahepatic cholostasis with low γ-GT in our hospital. We had tested the 27 exons of the BSEP gene of the 24 children by polymerase chain reaction and gene sequencing.

Results: 9 novel mutations were identified in 6 patients.

Conclusion: BSEP mutations play an important role in cases of progressive intrahepatic cholestasis with low γ-GT in eastern China.

PP-319

Molecular genetic analysis in eight Chinese patients with glycogen storage disease type III

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Objective: To get the mutation spectrum of glycogen debranching enzyme (AGL) gene in Chinese patients with glycogen storage diseases type III (GSD III).

Method: Genomic DNA samples were extracted from white blood cells of eight clinically diagnosed GSD III patients. 33 exons of AGL gene were analyzed by PCR, direct DNA sequencing, restriction fragment length polymorphism (RFLP) and GeneScan/SNaPshort analysis.

Results: All the patients have typical symptoms of GSD III: hepatomegalia, hypoglycemia, raised creatine kinase. While the variable clinical signs are muscle weakness and cardiomegalia. DNA sequencing of AGL gene revealed 8 patients to be compound heterozygote. We identified 10 different mutations, including 4 splicing mutations (IVS14+1G>T, IVS21+1G>A, IVS6-1G>A, IVS6+1G>A), 3 nonsense mutations (Y1428X, R977X, R469X) and 3 small deletions (c.2825delT, c.408-411delTTTG, c.2717-2721delAGATC)**.** Except for IVS14+1G>T and IVS21+1G>A mutations, all the others are novel mutation. The most prevalent mutation is IVS14+1G>T, accounting for 5 (31.25%) of 16 alleles examined. c.2825delT, IVS6+1G>A and IVS6-1G>A mutations were confirmed by RFLP. None heterozygote and homozygote of these 3 novel mutations was found in fifty normal controls by RELP. The two other small deletions were confirmed by GeneScan/SNaP short analysis.

Conclusion: IVS14+1G>T is a relative common mutation in Chinese GSD III patients. Combining with clinical and biochemical characters, the noninvasive molecular diagnosis for GSD III may replace the conventional means of enzymatic diagnosis that requires muscle and liver biopsy.

PP-320

The long term cultured of Human B cells from PBMCs stimulated via Soluble CD40 Ligand for the enhancement of antigen presentation in vitro

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We aimed to set up a method for long-term culture for B cells and to investigate whether B cells may be activated by soluble CD40 ligand (sCD40L) and loaded with virus peptide. B cells could be cultured in the presence of sCD40L and recombinant human interleukin-4 up to 50 days. The B cells in the S phase increased from 0% to 8.34%. The numbers of CD80, CD86, MHC classes I and II molecules on the sCD40L-activated B cells were significantly increased. Fluorescence microscopy showed that the activated B cells had strong fluorescence after peptide pulsing. Cytometry displayed that more than 98% scd40L-activated B cells were load by the hepatitis B core peptide. The results suggested the human B cells activated by sCD40L strongly up-regulated surface molecules on the B cells and had the ability to present antigen. Therefore, B cells may function as antigen presenting cells.

Poster Session – Transplantation

PP-321

Mycophenolic acid derivatives fail to improve renal function in post liver transplant patients with renal impairment

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Introduction: In orthotopic liver transplantation (OLT), mycophenolic acid derivatives avoid calcineurin inhibitor (CI)-induced nephropathy and improves renal function.

Aims and methods: To evaluate if mycophenolic acid derivatives would improve renal function in OLT recipients with renal impairment. Inclusion criteria: Adult OLT recipients, creatinine clearance test (CCT) <75 ml/min, stable graft function, rejection-free period of 3 months. Exclusion criteria: subjects with other renal diseases or gastrointestinal problems. Study subjects received either mycophenolate sodium 720mg or mycophenolate mofetil 1000mg orally BID, with 50% reduction or discontinuation of CI. Control subjects received CI. Primary endpoint was an increase in CCT at 2 years. Secondary endpoints were: side effects, rejection episodes and improvement in serum creatinine. P value < 0.05 was considered significant.

Results: Baseline characteristics were similar (study subjects n=8 - 3 mycophenolate acid monotherapy, 5 combination therapy; control subjects, n=10). At baseline, study subjects had a significantly lower median CCT (34.5 vs 48.4ml/min respectively,p=0.016) and a higher median serum creatinine than controls(166.5 vs 110.5μmol/L respectively,p=0.021). Within each cohort, the initial vs 2-year CCT was statistically similar (study p=0.779; control p=0.169). The change in CCT between both cohorts was similiar as well (p=0.248). Also, within each cohort, the initial vs 2-year serum creatinine was not statistically different (study p=0.310; controls p=0.813). The change in serum creatinine between cohorts was the same (p=0.374). There were no rejection episodes, however 75% of study patients on mycophenolate acid derivatives complained of gastrointestinal side effects.

Conclusion: Implementing mycophenolic acid derivatives in OLT recipeints with renal impairment, has made no improvement to their renal function.

PP-322

Hepatoprotective strategies against ischaemia reperfusion injury in fatty livers

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Hepatic steatosis is associated with increased operative morbidity/mortality from ischaemia-reperfusion injury (IRI) after liver resection or transplantation. We developed a dietary model of fatty liver disease in foz/foz (Alms1 mutant) mice with features resembling human metabolic syndrome and hepatic steatosis/steatohepatitis; we have shown that fatty livers in foz/foz mice are more susceptible to IRI than lean liver

Aims: (1) To study whether ischaemic preconditioning , or (2) administration of PPAR- agonist, Wy-14,643 in the diets of high fat diet (HFD)-fed wildtype (WT) and foz/foz mice 10 days prior to surgery, is hepatoprotective against IRI.

Methods: In mice subjected to partial hepatic ischaemia (60 min) followed by reperfusion (2-24hr), hepatic IRI was assessed by serum ALT and area of hepatic necrosis, and microcirculatory blood flow by in vivo microscopy.

Results: Ischaemic preconditioning was hepatoprotective in HFD-fed foz/foz or HFD-fed WT mouse livers against IRI (naive HFD-foz/foz ALT 15400 ± 7260* vs preconditioned HFD-foz/foz 740 ± 340 U/L, *p< 0.001). Administration of Wy-14,643 reversed HFD-induced steatosis and steatohepatitis; such PPAR- induced removal of hepatic fat conferred substantial hepatoprotection against IRI by serum ALT release and histology.

Conclusions: The mechanisms of IRI in fatty liver differ from those in lean liver; beneficial effects against IRI were derived from preconditioning or depleting steatotic livers of lipid by PPAR-enhanced fatty acid disposal. These findings open the opportunity to prevent IRI in human fatty liver before surgery or organ donation.

PP-323

Preliminary investigation on immune microenvironment for reestablishment of immunity to hepatitis B after liver transplantation

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Objective: How to reestablished active immunity to HBV after liver transplantation involved many factors.Current reports mainly focused on clinical indications,while,put less concentrations on immunoenvironment.In fact,recovery of immune microenvironment is likely the first step for achineving immune response.IDO,as a suppressive immunomodulatory enzyme,could compromise immune response to vaccine.Here,we investigated the role of IDO in vaccination response.

Methods: 25 recipients,given informed consent in advance,average time after LT was 33.32±14.81M(11-77M).They were prospectively vaccinated with Engerix-B containing 40 ug HBsAg up to 4 times at monthes 0,1,2,6 (cycle1)under continuous LAM and/or HBIG prophylaxis.Another 25 normal control were not given any interference.Fast blood were collected at pre-vaccination,1W after 1th and 3th vaccination respectively.Tryptophan and kynurenine were measured by HPLC,for estimating IDO activity,the kynurenine to tryptophan ratio was calculated.In parallel,HBV markers,HBV DNA and HBsAb quantification were tested.

Results: 23 recipients had completed recorders at the end point.35%(8/23) had positive vaccination response, average HBsAb titer increased form 75.675IU/L (28.370-147.600IU/L) to 184.224IU/L(59.190-668.500 IU/L) after finishing the schedule.Mean IDO activity were slightly higher in all recipients at pre-vaccination(51.125 micromol/mmol in positive group,54.040 micromol/mmol in negative) compared to normal control (48.493micromol/mmol) (P>0.05).Mean kynurenine(3.394 micromol/L in positive group,3.755 micromol/L in negative) and tryptophan (66.159 micromol/L in positive group,72.265 micromol/L in negative) level were singnificant higher than normal control (2.664 micromol/L in kynurenine, 53.401micromol/L in tryptophan) (p<0.05).

Conclusion: Positive response recipients had a low or nearly normal level of IDO and kynurenine probably contributed to good vaccination.IDO and kynurenine suppressed immune response were one of the factors to poor vaccination.IDO and kynurenine decrease with tryptophan increase suggest immuno-microenvironment recovery and facilitate to vaccination after liver transplantation.

PP-324

MicroRNA signatures of small size liver graft in rat recipients

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Background: Living donor liver transplantation (LDLT) is an evolving approach to ease the severe scarcity of donor livers. Small size liver graft transplantation (SSLGT) in rats is a useful model to investigate graft function, growth and survival in LDLT. Molecular mechanisms underlying the graft growth are poorly understood. miRNAs are noncoding short RNA species that negatively modulate expression of genes controlling cell differentiation, proliferation and apoptosis. The present study aims to identify critical miRNAs affecting small graft growth and function.

Methods: SSLGT at 95, 75 and 45% graft volume was performed in rats. Two days after the transplant, RNA was extracted from graft tissue. miRNA expression profile was analyzed with bioarray chips, and potential miRNA targets were determined by bioinformatics software.

Results: Comparing control liver, four miRNAs were up-regulated in small size grafts; miR-223 was increased nearly 6-fold in 75% volume graft. Whereas, 37 miRNAs were down-regulated from 3 to 10-fold, and miR-122a, Let-7b and miR-26a were reduced by more than 90% in 45% volume grafts. In general, the smaller the graft size transplanted, the lower was the expression of these miRNAs. The up-regulated miRNAs exhibited sequence homology with genes negatively involved in cell proliferation. However, potential target genes for down-regulated miRNAs are those which promote differentiation, mitosis, signal transduction and metabolism, such as a group of growth factors, STAT3, glycogen synthase, cyclins, MAP kinases, calmodulin, etc.

Conclusion: Down-regulation of critical miRNAs in SSLGT appears to play a central role in promoting the growth of small size grafts.

PP-325

Intrahepatic bile ducts and its microcirculation after warm hepatic ischemia-reperfusion injury in the rat

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Background: The warm ischemia-reperfusion injury is one of the most important factors for Ischemic-Type Biliary lesion，which is called The Achille's heel revisited. Differenting from hepatocyte, intrahepatic bile ducts mainly supplied from peribiliary vascular plexus (PBP), which originate from branches of the hepatic artery。So we investigated what the pattern of PBP during the warm ischemic-reperfusion injury and regeneration of intrahepatic bile ducts.

Medthods: The liver was harvested at 0h, 6h, 24h, 3d, 7d, 14d and 30d after rat re-arterialized orthotopic liver transplantation following 0(W0 group) or 10(W10 group) minutes warm ischemia-reperfusion. A series slices had immunostained by CK19, vW factor, PCNA, VEGF-A or investigated the VEGF-A mRNA by in situ hybridization.

Results/Conclusions: The endothelial cells of peribiliary vascular plexus of W10 group were more seriously swell, necrotic and apoptotic, and there were micro-thrombosis in microvessels. These resulted in the obstruction of microcirculation of bile ducts. An immediate proliferative response of intraportal bile ducts were followed by proliferation of the microvascular compartment after warm ischemia-reperfusion. These data supported that bile ducts is more susceptible than hepatocytes during warm ischemia-reperfusion injury. The staining of VEGF-A and VEGF-A mRNA of cholongiocytes were stronger in W10 group at 3 days to 14 days after reperfusion as compared with the W0 group and hepatocytes. So the VEGF-A secreted from cholongiocytes is one of the important growth factors to promote the proliferation of bile ducts and PBP.

PP-326

N-O donating drugs improve the distribution and engraftment of transplanted hepatocytes in mouse liver

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Introduction: Hepatocytes transplantation is a promising therapy for genetic hepatic disorders caused by hepatocyte dysfunction and currently treated by liver transplantation.

This therapy is still limited by the loss of many transplanted cells in the portal radicles before their entry into the sinusoids to engraft.

Methods: In this study we examined the vasodilator effect of Glyceril-trinitrate (GTN) on hepatic sinusoids and assessed its efficiency on hepatocytes engraftment in a syngenic mice model. We first assessed the effect of GTN portal infusion on the parenchymal spreading of colored microspheres. Hepatocytes transplantation in a syngenic mice model was then performed concomitantly with GTN infusion. The distribution of transplanted hepatocytes and their ultimate engraftment were analysed.

Results: After GTN perfusion, 27% of microspheres shifted from the portal to the sinusoidal zone. Transplanted hepatocytes distribution changed significantly in the portal and parenchymal zones from respectively 53 ± 2% and 46.8 ± 2 % in control animals to 32.5 ± 2.4% and 67.5 ± 2.4% in GTN-treated animals. At days 7 and 15, we noted a significantly better engraftment in GTN group vs. controls (60 ± 4 vs. 37 ± 2 transplanted hepatocytes in 20 fields x40).

Conclusions: Portal perfusion of GTN improved the access of microspheres and transplanted hepatocytes to the sinusoidal bed. This probably explains the better engraftment of transplanted hepatocytes. These results suggest that drug dilatation of portal pedicles prior to transplantation increases the efficiency of hepatocyte grafting.

PP-327

The significance of hepatic stellate cells activation on small-for-size fatty liver graft injury

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Background and Objective: Fatty liver used in living donor liver transplantation (LDLT) may be more susceptible to ischemia-reperfusion injury. In this study, we aimed to investigate the significance of HSCs cell activation on small-for-size fatty liver graft injury and the underlying molecular mechanisms in a rat liver transplantation model.

Materials and Methods: A rat non-arterialized orthotopic liver transplantation model using fatty liver grafts and cirrhotic recipients was used. Liver architecture and ultrastructure change related to hepatic sinusoidal injury were checked at different time points. HSCs activation was detected by immunostaining. cDNA microarray screening was employed to compare gene expression profiles among whole normal graft, whole fatty graft, small-for-size normal graft, and small-for-size fatty graft in order to identify distinct gene panel linking to small-for-size fatty liver graft injury.

Results: The early and significant activation of HSCs in small-for-size fatty grafts was well correlated with progressive hepatic sinusoidal damage as well as survival rates. Among the over-expressed genes screened by cDNA microarray, Wnt4 was upregulated more than 10-fold in small-for-size fatty liver graft compared with whole fatty graft and small-for-size normal graft and the same significant change was detected at protein level. Overexpression of other Wnt family genes and their receptors were also investigated in small-for-size fatty liver grafts.

Conclusions: Significant activation of HSCs in small-for-size fatty liver grafts suggests its important role in acute phase graft injury. Upregulation of Wnt4 expression in small-for-size fatty liver graft implicates a possible relationship between Wnt4 signaling pathway and HSCs activation.

PP-328

Assessment of HepaHope bioartificial liver (BAL) system for treating liver failure patients

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A large number of patients die annually from liver failure worldwide. HepaPheresis System is an extracorporeal BAL system to treat such liver failure patients as a potential life-saving alternative. The BAL contains porcine liver slices with multiple, native cell types in well-preserved structures that allow efficient biochemical function and removal of toxins from patients' blood. Safety of source material has been examined. Source pig livers were free of potential zoonotic infectious agents such as swine hepatitis E virus, porcine reo, rota and adenoviruses. PERV RNA was present three out of eight pig livers, but co-culture assay for the detection of infectious PERV showed no PERV infection on 293 human embryonic kidney cells. Several *in vitro* and *in vivo* studies have been conducted to evaluate safety and efficacy of the system. The liver slices in the system were biologically functional by clearing ammonia (60-80%) and lidocaine (60-70%) and accumulating their metabolites (BUN and DMX). In pre-clinical safety study of large canine (>20kg) study, all animals (8 dogs) survived the treatment without major clinical complications. There were no adverse immune reactions (canine IgG/IgM and inflammatory cytokines, IL-10 and IFN-γ). PERV RNA was not detected in all animals. Pre-clinical efficacy data utilizing a canine acute liver failure model showed statistically significant ($P<0.05$) outcome in survival and other biomarkers (lactate, K, pH, phosphorous) with improvement in intracranial pressure. These favorable pre-clinical study results and no zoonotic infectious agents in source animals have encouraged feasibility studies in human patients with liver failure.

PP-329

Changes of spleen morphology and immune function of hepatic cirrhosis rats after liver transplantation

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Objective: The purpose of this study was to investigate the changes of spleen morphology and immune function of hepatic cirrhosis rats after liver transplantation.

Methods: Hepatic cirrhosis model was established by subcutaneous injections of carbon tetrachloride. Liver transplantation model was established with two-cuff technique. The portal vein pressure, morphological changes of spleen were observed before and after liver transplantation in hepatic cirrhosis rats. The spleen T lymphocyte subsets and the serum tuftsin level before and after liver transplantation were assayed by using flow cytometry, high performance liquid chromatography (HPLC), respectively. The level of TNF, NO of portal vein were determined by Kit.

Results: Before liver transplantation, portal vein pressure and spleen index were great increased; pathological sections of spleen showed that white pulp areas were decreased while the spleen trabecula areas were increased. Meanwhile, the rate of CD4/CD8 of spleen T lymphocyte subsets and the level of serum tuftsin were significantly decreased. And ，the level of TNF, NO were significantly increased. After liver transplantation, portal vein pressure and spleen index were significantly decreased; pathological sections of spleen showed that white pulp areas were increased, and spleen trabecula areas were decreased. Meanwhile, the rate of CD4/CD8 of spleen T lymphocyte subsets and the level of serum tuftsin was increased. And ，the level of TNF, NO were significantly decreased.

Conclusions: Portal vein hypertension of hepatic cirrhosis rats could be relieved after liver transplantation. Liver transplantation can improve the impaired immune function of spleen through downregulating the level of TNF and NO.

PP-330

HBV may have transformed to be potential opportunistic virus after liver transplantation

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Objective: Liver transplantation (LT) outcome had been reduced by HBV reinfection/HB recurrence. PBMC HBV DNA, persisting positive even undergoing LAM and HBIG therapy, is considered the major source of reinfection/recurrence. We aim to clarify why HBV DNA can persist in PBMC post-LT and its clinical significance.

Methods: 25 HBV related LT recipients were enrolled. The post-LT time was 29.62±21.06 months and age was 44.7±9.4 years. They all have administered LAM and HBIG after LT. 9 cases were negative for sera HBV DNA pre-LT and 17 were positive with titer of 6.11±1.41 Ig10 copies/ml. Vein blood samples and bone marrow was obtained with written content. PBMC, T, B, monocyte (MNC) and CD34+ cell were isolated by Ficoll-Hypaque density gradient centrifugation and MASC. Anti-HBs was quantified using electrochemiluminescence immunoassay. HBV markers were detected using ELISA. HBV DNA and cccDNA titer in serum, PBMC, T, B lymphocyte, MNC and CD34+cell were detected using real-time FQ-PCR.

Results: 25 cases are all positive for anti-HBs with mean titer of 104.34±99.71IU/L and are normal liver function. Sera HBV antigens of all cases are negative. Anti-HBe and anti-HBc are positive in 8 cases, anti-HBc is positive in 9 cases, anti-HBe and anti-HBc are negative in 8 cases. All sera HBV DNA are negative in 25 cases. PBMC, MNC, B, T and CD34+ cell is all positive for HBV DNA with titer of 3.326±0.6596, 2.9425±0.6462, 2.2713±0.6223, 2.9522±0.9319, 3.3373±0.6583 Ig10 copies/106 cells respectively. There are no HBV replicative evidence in CD34+ cell and immunocyte.

Conclusion: HBV DNA positive in CD34+ cells maybe a key factor of HBV DNA persisting in immunocyte, furthermore, HBV may have transformed to be characteristic of potential opportunistic virus after liver transplantation.

PP-331

Continuous graft monitoring with microdialysis early after liver transplantation

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Aim: The objective is to permit continuous monitoring of metabolic changes in the transplanted liver at the bedside.

Methods: Three consecutive patients undergoing whole-organ orthotopic liver transplantation were studied.At the end of the operation intrahapatic implantation of a microdialysis catheter was placed in segment Ⅳ in the direction of segment Ⅷ at the level of falciform ligament.Consecutive serial samples were collected at 1-h intervals after the operation.Sampling was continued until the patient went out of the ICU, about 5-7days.Glucose, lactate,pyruvate and glycerol concentrations were measured. Meanwhile patients did some routine examinations and were observed continually. Besides, these patients were followed up after they were discharged.

Results: In one patient,the glycerol level increased rapidly for two times during 48h after the operation,one time with a rapid increase in lactate.After adjusting the dose of immunosuppressant,the level of glycerol begun to decrease.In the other two patients,the glycerol level increased during the second 24h after the operation,and 12h later.it reached the peak.One of the two complicated jaundice six months after the operation.The complications of the bile duct resulted from the ischemia-reperfusion injury was considered.

Conclusions: The procedure of microdialysis is easy to perform and safe.It allows continuous monitoring of tissue metabolism in the transplantation graft.The rapid increase of the glycerol level two or three days after the operation may indicate the acute rejection of the transplanted liver.

PP-332

Low dose calcineurin inhibitor and mycophenolate mofetil therapy after liver transplantation is Nephro- and hepatoprotective and may also promote allograft tolerance

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Background: Calcineurin inhibitor (CNI)-related nephrotoxicity significantly contributes to chronic renal failure after liver transplantation (LT).

Methods: In this prospective study, LT patients with renal dysfunction were randomized to either receive mycophenolate mofetil (MMF) followed by stepwise reduction of CNI with defined minimal CNI-trough levels (MMF group) or to continue their maintenance CNI dose (control group). Immune monitoring was performed in a subgroup of the patients.

Results: In the MMF group (n=50), renal function assessed by serum creatinine improved >10% in 62% of patients, was stable in 36% and deteriorated >10% in 2% after 12 months compared with baseline values. Mean serum creatinine levels (+/- SD) significantly decreased from 1.90+/-0.44 mg/dL to 1.61+/-0.39 mg/dL and the corresponding calculated glomerular filtration rate significantly increased from 48.7+/-14.1 mL/min to 57.6+/-16.9 mL/min over a 12-month follow-up period. Blood presssure and levels of liver enzymes significantly decreased. In the control group (n=25), there were no significant changes with respect to the investigated parameters. The MMF group had significantly lower numbers of circulating cytotoxic T cells compared with the controls; whereas regulatory T cells significantly increased. Conclusions: Combined MMF and minimal dose CNI therapy after LT is nephro- and hepatoprotective, and may also promote allograft tolerance.

PP-333

Management of middle hepatic vein in living donor liver transplantation of right liver grafts

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Objective: The aim of this study was to summarize the factors that influence the resection of middle hepatic vein in living donor liver transplantation of right liver grafts.

Methods: From June 23, 2006 to November 15, 2007, 19 right lobe LDLTs were performed in our centre for adult patients. Of these, 8 patients received a right lobe with MHV graft (42.1%). The decision to take MHV with the graft was made based on the donor-to-recipient body weight ratio (in 4 cases) and the size and the number of the MHV tributaries from the anterior segment (in another 4 cases). Donor and recipient characteristics, operation time, blood loss, hospital stay, postoperative liver function and complications were compared.

Result: All of the donors survived from the operation and no serious complications occurred. There was no significant difference in operative outcomes and postoperative liver function ($P > 0.05$) between these two groups. With regard to recipient, there was no significant difference in operation time and blood loss between these two groups while the hospital stay was significantly shorter in the right lobe with MHV graft group($P<0.05$). As to liver function tests, there was no significant difference in ALT or PT levels between these two groups while serum bilirubin clearance was much delayed in the right lobe without MHV graft($P<0.05$).

Conclusion: LDLT using a right lobe liver graft with MHV is safe to the donors and beneficial to the recipients. The major factors that influence the graft selection are volumetric factors and anatomical factors.

PP-334

Idiopathic hyperammonia post orthotopic liver transplantation

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Idiopathic hyperammonemia is a rare but frequently lethal complication of solid organ transplantation. It is characterized by abrupt alteration of mental status and markedly elevated plasma ammonia level in the absence of obvious liver diseases. It often results in intractable coma, brain edema and death.

We report a case of acute idiopathic hyperammonemia 7 years post liver transplantation for autoimmune hepatitis. This is a 37-year-old female presented with sudden-onset severe headache with rapid deterioration of mental status and became comatose within 2 hours. Initial laboratory results revealed markedly elevated serum ammonia level (>1000 μg/dl), with moderate elevation of ALT, normal prothrombin time. The patient was intubated and admitted to intensive care unit. She was treated with ammonia trapping therapy with sodium benzoate and arginine and later started with continuous veno-venous hemodialysis. She developed brain edema and seizures on hospital day 2. She was found to be brain dead on hospital day 6.

In summary, idiopathic hyperammonemia post solid organ transplantation is rare. There were less than 10 cases reported, which were all in lung and/or heart transplantation. To our knowledge, this is the first case report of idiopathic hyperammonemia post liver transplantation. The reported morality rate was 67%. The only reported surviving case was treated with aggressive early hemodialysis and ammonia trapping therapy with intravenous sodium phenylacetate, sodium benzoate and arginine. Therefore, early identification of idiopathic hyperammonemia post solid organ transplantation by checking serum ammonia levels in patients with neurological symptoms is critical for early diagnosis and prompt therapy.

PP-335

The question of the relationship between expression of aquaporin-4 and ischemia-reperfusion injury of rat liver

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Biliary complications was to be found in the operations of liver transplantation, liver IRI is the important link of Primary non-function and Functional cholestasis. The pathology and physiology mechanism of PNF is concentrated on active oxygen, etc, have deficient congnition on aquaporins in this process. So this investigation was raised by built rat liver IRI model , study the changes of aquaporin-4 and the relationship between the changes of aquaporin-4 and the liver function in this process, hope probe the etiopathogenesis of the liver IRI and functional cholestasis in a new way.

Method: Raise the rat liver IRI model. Divid the experimental animal in control and ischemia-reperfusion groups pairing-randomly. Keep the blood serum of model rat and kill the rats in different times, keep the serum specimen to liver function detection. Keep the liver collection to HE coloretur、immunohistochemistry and RT-PCR examination.

Results: 1. The DB, IB in the ischemical reperfusin injury group increase obviously .2. The changes between IRI and sham-operation rat liver sections by anti-AQP4 immunohistochemistry coloretur: The contrast in the IRI group, between IRI and sham-operation group have statistically significance.4. The mRNA expression level of AQP-4 in IRI rats liver is manifest cut down compared to sham-operation rats.

Conclusion: 1.IRI is one of the motivation of acute liver failure and functional cholestasis.2.The changes of AQP-4 expression maybe the important indexes of liver function, take all-important effect in the pathology and physiology process of liver ishemical reperfusion injury.

PP-336

Protective effect of ischemic preconditioning in graded steatotic livers subjected to ischemia/reperfusion injury in mice model

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Background: Hepatic ischemia/reperfusion (I/R) is a major risk factor for poor outcomes in liver surgery. Ischemic preconditioning (IPC) has been shown to protect lean and even steatotic livers from I/R injury. Whether IPC could protect any grade of steatotic livers from I/R injury remains obscure.

Methods: A mouse model of steatosis induced by methionine/choline deficient diet was established. The effect of IPC was assessed in different grade of steatotic livers subjected to 75 minutes of ischemia and 2 hours of reperfusion. Hepatic ATP metabolic characteristics of these mice were also studied.

Results: Lean, mild, moderate and severe grade of hepatic steatosis was successfully established. Studies of serum aminotransferases showed that 75 min of ischemia and 2 h of reperfusion caused significantly hepatic injury, and the severity of hepatic injury was correlated with the grade of steatosis. IPC significantly reduced I/R injury in lean and mildly steatotic livers. In contrast, the protective effect was lost in moderately and severely steatotic livers. Histological data further confirmed these findings. IPC significantly preserved ATP contents in lean and mildly steatotic livers subjected to I/R injury, meanwhile could not preserve ATP contents in moderately or severely steatotic livers. Further study showed that IPC could not up-regulate hepatic ATP synthase beta subunit (ATP5B) in steatotic livers.

Conclusions: The protective effect of IPC was lost in moderately and severely steatotic livers in our mice model. This finding indicated that IPC may be not suitable for moderately and severely steatotic livers.

PP-337

Oncosis represents the main type of cell death during hepatic ischemia-reperfusion injury in cirrhotic rats

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Objective: To research on the main type of hepatic cells death during hepatic ischemia-reperfusion (I/R) injury in cirrhotic rat.

Methods: Primary method involves implementation of carbon tetrachloride replication to duplicate a model of cirrhotic rats, and then randomly group them into Sham group and I/R group. The I/R group is further divided into five subgroups: 0 h, 1 h, 6 h, 24 h, 48 h. The samples of livers will undergo several biochemical comparisons, including serum ALT/AST level, the Na^+-K^+ ATPase, Ca^{2+}ATPase, flow cytometry observing percentage of apoptotic/ oncotic hepatic cells, and observation of changes in hepatic cellular structures under transmission electron microscope.

Results: In comparison to the Sham group, the I/R group shows a significant increase in the level of serum AST and ALT, with the peak at 6 hours followed by gradual declination. The active levels of the Na^+-K^+ ATPase and Ca^{2+}ATPase dramatically decrease to minimum one hour after reperfusion treatment and later with a recovery. Hepatic cells suffer oncosis in 6 hours of the early period, while they suffer apoptosis at late stage around 24 hours after reperfusion. Typical appearances of oncosis and apoptosis can be observed.

Conclusion: Oncosis represents the main type of cell death during I/R injury in cirrhotic rat, the severity of hepatic injury correlates with the oncosis.

PP-338

Screen serum biomarkers with relation to the recurrence on the patients with liver transplantation for hepatocellular carcinoma Pre-operation

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Objective: To appraise and compare protein expression profiles in sera of patients with or without recurrence in the liver transplantation patients for hepatocellular carcinoma (HCC) within *Shanghai Fudan criteria* using SELDI-TOF-MS technology.

Method: A cohort of pre-operation serum samples were obtained from 17 patients with recurrence and also 29 patients with disease free survival (DFS) in a 3-year follow-up study. Special protein spectra were determined by weak cation exchange (WCX) chip measurement and the protein fingerprints between two groups were compared by Biomarker Wizard software package.

Result: According to serum protein fingerprints, a total of 331 protein peaks were identified at the M/Z value ranging from 2000 to 30000. 7 significant differential proteins were found between the groups of HCC with recurrence and those with DFS（P<0.01）. 5 proteins were up-regulated with the M/Z value of 2143、2353、2577、5070 and 3518 in recurrence group; 2 proteins were down-regulated with the M/Z value of 22874、22767 in recurrence group.

Conclusion: These discriminated proteins may play the key role in predicting the prognosis of the patients undergoing liver transplantation for HCC.

PP-339

The virological factors related to the recurrence of hepatitis B after orthotopic liver transplantation: YMDD mutant induced later recurrence

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Background: There are a lot of factors related to recurrent hepatitis B after orthotopic liver transplantation (OLT). We aimed to investigate the role of virological factors in them.

Methods: Clinical data, especially virological and histological data of patients with recurrent hepatitis B after OLT were analyzed.

Results: In a follow-up ranged from 3.07 to77.97 months, 18 patients relapsed in 230 (range: 17 to 708) days post-OLT. The peak viral level averaged as high as 7.20±2.6 log copies/ml. The HBeAg status, YMDD mutant pre-OLT didn't correlate with the time to recurrence, HBV DNA peak level, or level of liver enzymes after recurrence. But patients who relapsed earlier than 90 days post-OLT showed a higher HBeAg positive rate before transplantation and a higher viral level pre- and post-transplantation, than those who relapsed later. The YMDD mutant rate after recurrence in patients who relapsed later than 180 days after transplantation was significantly higher than in those who relapsed earlier (9/11 vs. 1/6, χ^2=6.804, P=0.009). Excessively expressed HBV markers in hepatocytes and two cases of fibrosing cholestatic hepatitis were observed. Most patients (11/13) recovered after effective anti-HBV therapy.

Conclusions: The cytopathic effect of HBV played an important role in the pathogenesis. The positive HBeAg and high viral load pre-OLT may related to earlier recurrence (<3 months post-OLT). The presence of YMDD mutants after recurrence related to later recurrence (>6 months post-OLT). The anti-HBV therapy is the key point of therapy, and nucleotide analogs with lower resistance will be the choice.

PP-340

Coronary artery calcification scores in the assessment of patients for liver transplantation: association with other cardiovascular risk factors.

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Background and Aims: Patients with advanced liver disease are at increased risk of cardiovascular events, especially following orthotopic liver transplantation (OLT).Coronary artery calcification (CAC) is a novel and independent predictor of cardiovascular risk but its prevalence and utility in patients with cirrhosis is unknown. The aim of this study is to define the prevalence of CAC and its association with markers of disease severity and standard measures of cardiovascular risk, in a large cohort of patients undergoing OLT assessment.

Methods: Single centre prospective observational study. CAC scores were derived using the Agatston method from thoracic computed tomography scans and correlated with cardiovascular risk factors and measures of liver disease severity.

Results: 101 patients (66 males) with mean age of 53.2 years. The mean CAC score was 292 HU (range 0-3533). Correlations were identified between CAC score and age (r=0.477; p<0.001), male sex (r=0.262 ; p=0.008), family history of cardiovascular disease (r=0.208 ; p=0.036), Framingham risk score (r=0.621; p<0.001), MELD (r=0.221; p=0.027), systolic (r=0.285; p=0.004) and diastolic blood pressure (r=0.267; p=0.007), Cytomegalovirus status (r=0.278 ; p=0.005), fasting glucose (r=0.330; p=0.001), number of coronary vessels involved (r=0.899 ; p<0.001), and components of the metabolic syndrome (r=0.226 ; p=0.026). After multivariate analysis; age, systolic blood pressure, fasting glucose, number of features of metabolic syndrome and number of vessels involved remained significantly associated with CAC.

Conclusion: This study identifies the high prevalence of occult coronary artery disease in patients undergoing OLT assessment and identifies a strong relationship between CAC scores and a limited number of specific cardiovascular risk factors. The usefulness of these factors in predicting peri and post-operative cardiovascular events in patients undergoing OLT requires prospective evaluation.

PP-341

Novel monitoring of cell mediated immunity (CMI) with Immukow® , induction with Basiliximab and delayed Calcineurin (Cni) introduction rapidly corrects renal dysfunction, avoids rejection and mortality in sick liver transplant recipients

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Risk of death from sepsis and renal failure post LT can be reduced by Immune monitoring early post LT. Immukow® is a useful guide in measuring CMI and monitoring immunesuppression (IS). Early post-liver transplant (LT) renal dysfunction (RD), secondary to calcineurin inhibitors (Cni), is associated with post-LT death. Methods: From 2003 to 2007, 265 patients received liver transplantation at NZTI. 55 (20.8%) of them had early post-liver transplant renal dysfunction (the average GFR = 29.4 (11 - 56) mL/minute/1.73 m²). Induction with Basiliximab, steroids with Mycophenolate mofettil (MMF) and delayed timing of Tacrolimus (Tac) based on the Cylex® IM Immukow ® value of >200pg/ml.

Results: 4 weeks after LT, the average GFR of these patients was 52.9 (20 - >60). 32 (58.2%) has complete recovery of RF (GFR >60 vs. 32.8), 22 (40.0%) partially recovered RF with mean GFR 43.9 vs. 24.8, and only 1 (1.8%) patient still had poor RF. Acute cellular rejection (ACR) was found in 8 patients (14.5%) (mild-moderate: 2; moderate: 6) with only 4 (7.27%) in the first month during this protocol. At 4 year follow up only 5 (9%) died with RD including those with complete or partial recovery of RF. Survival with early RD was 87.2% at 1 year, 82.6% at 3 and 71.9% at 4 years.

Conclusion: Measuring CMI using Immukow ® helps avoid over and under IS when multiple agents are used and timed delayed introduction of Cni in a low-dose TAC protocol rapidly corrects early post-LT GFR, with low ACR and mortality.

Poster Session – HBV

PP-342

Study on the number and function of circulating CD11c+ myeloid dendritic cells of patients with Chronic Hepatitis B

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Background: Dynamic interactions between the hepatitis B virus (HBV), hepatocytes and the host immune system may contribute to the viral persistence and disease progression. $CD11c^+$myeloid dendritic cells, which believed to evoke preferentially T cell responses, orchestrate the innate and adaptive immunities against invading pathogens.This study aims to evaluate the frequency, immune phenotype and function of $CD11c^+$mDCs in patients with chronic hepatitis B, and to explore the influence of mDCs on persistent HBV infection.

Methods: Peripheral blood was collected from 37 chronic hepatitis B patients, and 21 healthy blood donors used as controls · Flow cytometry was used to analyze the frequency of circulating $CD11c^+$mDC and its expression of co-stimulatory molecules. mDC isolated by immunomagnetic selection were incubated and induced in vitro using PolyI:C. The stimulatory capacity of mDC was determined in allogenic mixed leukocyte reaction (MLR). The supernatants were also measured for IL-12 production using ELISA method.

Results: Peripheral mDC frequency in patients with chronic HBV infection significantly decreased compared with healthy controls (p<0.01). Respectively, mDC frequency inversely correlates with ALT level (r=-0.410, p<0.05) and HBV DNA level (r=-0.481, p<0.01). Moreover, among which 15 patients have undergone liver biopsy simultaneously , an inverse correlation was also found between mDC frequency and their inflammation levels(r=-0.521, p<0.05). Both CD80 and CD86 expression on freshly isolated mDC surface are lower. However, when compared with health controls, CD86 in patients is relatively higher (p< 0.05). The stimulating capacity in MLR (p<0.05) and IL-12 secretion level (p< 0.001) of mDC in HBV patients were much lower than that of normal controls.

Conclusions: The decreased number and impaired function of $CD11c^+$ mDC might result in inability of host immune system to effectively clear HBV and lead to viral persistence and disease chronicity.

PP-343

The modulation of Thymosin alpha 1 in the maturation, differentiation and function of human peripheral blood derived dendritic cells in HBV patients.

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Background: Dendritic cells (DCs) play an important role in the induction of T-cell responses. We hypothesize that the hampered antiviral T-cell response in chronic hepatitis B patients is a result of impaired Dendritic cells (DCs) function. Thymosin α 1(T-α1) is a biological response modifier. Previous studies show that T-α1 has abilities to affect the maturation, differentiation, and function of T-cells. It has been used clinically for the treatment of chronic HBV. However, little is know about what action T-α1 has on DCs. The study aims to evaluate the effect of T-α1 on peripheral blood derived DCs maturation, differentiation, and function in HBV patients.

Method: Peripheral blood was collected from 18 chronic hepatitis B patients, and 12 healthy blood donors used as controls. DCs were generated by culturing peripheral blood mononuclear cells with GM-CSF, IL-4 and TNF-α in vitro, with or without T-α1 in different levels (0.5μg/ml and 0.2μg/ml). The FACS was employed to detect the expression of surface markers on DCs. The stimulatory capacity of DCs was determined in MLR. The supernatants were measured IL-12 production by ELISA

Results: Compared with normal controls, the expression of CD80, CD86, HLA-DR were much lower in patients (P<0.001), so as MLR (P<0.01) and IL-12 secretion level (P< 0.001). Both in patients and normal controls, T-α1, especially at 0.5μg/ml, increased the expression of CD80 (P=0.043), CD86 (P=0.036),HLA-DR(P=0.025). MLR test also showed DCs added with T-α1 the proliferation of T cells stimulated were much higher than blank(p<0.05).

Conclusion: T-α1, especially at 0.5μg/ml, promotes the differentiation/activation of DCs in HBV patients. T-α1 may be valuable for the generation of active DCs to be as vaccination strategies of HBV patients.

PP-344

Study on the expression of toll-like receptor 3 on dendritic cells derived from peripheral blood monocyte of chronic hepatitis B patients

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Objective: To investigate the expression of Toll-like receptor 3 (TLR3) on dendritic cells (DC) derived from peripheral blood mononuclear cells (PBMC) of chronic hepatitis B (CHB) patients and to explore the mechanism of sustained infection of hepatitis B virus (HBV).

Method: Twenty CHB patients were randomly screened in the study, and ten healthy persons were as controls. The monocytes isolated from peripheral blood of candidates were incubated with rhGM-CSF and rhIL-4 to induce the DCs generation and proliferation. Then the phenotype of DCs was identified by microscope. The expression of the phenotypes (HLA-DR, CD80, CD86, CD83) of immature and mature DCs were characterized by flow cytometer. Furthermore, the expression of TLR3 on mDC and immature DCs (imDC) were determined by flow cytometry and Western blot analysis respectively.

Results: As for health volunteer, the expressions of CD80, CD86, HLA-DR and CD83 on DCs at the 7th day, which were 82.35±8.67%、79.61±10.08%、92.79±8.48% and 83.76±5.47% respectively，was significantly higher than that at the 5th day which were 28.31±8.79、31.17±11.23、27.61±10.28 and 23.46±11.53 respectively (P<0.001). However, no difference was showed between mDC and imDC of the CHB patients groups (P>0.05). The expression of TLR3 on imDC was significantly higher than that on mDC at control group (P<0.001), but not significant difference was showed at CHB patients (P>0.05). And the expression of TLR3 on imDC at CHB patients group revealed significant lower than that of controlled group (P<0.001)

Conclusions: The expression of TLR3 on peripheral DCs of CHB patients was decreased compared to that of healthy persons, it might be one of the important pathogenesis of the HBV sustained infection.

PP-345

Safety and efficacy of 52 weeks of adefovir dipivoxil in Chinese HBeAg- Chronic hepatitis B

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Background: HBeAg negative chronic hepatitis B (CHB) is an increasing problem worldwide, including in Asia. Clinical experience suggests that these patients require long-term nucleos(t)ide administration. This is the first study reported for adefovir dipivoxil 10mg in Chinese HBeAg negative CHB.

Methods: In a multicentre, open-label study, 533 HBeAg negative CHB subjects were enrolled to receive ADV for 2 years. HBV DNA was performed using Cobas Taqman assay (LLD <200copies/ml).

Results: 522 patients completed 52 weeks of treatment. At baseline the median ALT and median HBV DNA were 1.9 x ULN and 6.6 log10 copies/mL, respectively. At week 52 median ALT was 0.6 x ULN, with 82% (426/522) achieving serum ALT normalization. Mean HBV DNA reduction from baseline to week 52 was 3.53 log10 copies/mL, with a median HBV DNA of 2.5 log10 copies/mL at week 52, and 79% (413/522) achieved undetectable HBV DNA (<300 copies/ml). No subject achieved HBsAg loss/HBsAg seroconversion. There were no SAEs related to ADV, and no AEs leading to discontinuation of ADV. Conclusion: ADV for 52 weeks in HBeAg negative CHB was effective in inhibition of HBV replication and ALT normalization. ADV was safe and well tolerated. The study continues for a second year.

PP-346

HBV polymerase inhibits Poly (I:C) and NDV induced production of β-interferon in human hepatocytes

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Although researches have been undertaken to identify the key mediators of responsiveness to IFN-α, mechanisms underlying defective responses to IFN or defective production of IFN in chronic hepatitis B patients have not been fully elucidated. In this study, we

investigated if the production of interferon in HBV replicating hepatocytes was impaired and its molecular mechanisms. By using co-transfection of Huh7 cells with a HBV-replicative plasmid and Poly (I:C), we found that, compared to control, β-interferon produced from HBV replicating cells was significantly decreased. Besides, HBV replication also inhibited the production of β-interferon induced by NDV. To identify the responsible viral proteins, HBV gene products including polymerase, core and X protein were transfected respectively into Huh7 cells with Poly (I:C). Results showed that HBV polymerase, neither core nor X protein, inhibited production of β-interferon. To further study the underlying mechanism, TBK1/IKKε reporter plasmids and pHBV3.8 were co-transfected into Huh7 cells. Results showed that HBV replication significantly inhibited TBK1/IKKε activated interferon promoter activity. Further studies are being carried out to identify the principal transcription factors downstream of TBK-1/IKKε targeted by HBV polymerase to inhibit IFN induction. Taken together, our findings suggested a novel role of HBV polymerase in inhibition of interferon production in human hepatocytes.

PP-347

Characterization of HBx protein complex by TAP/MS

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Hepatitis B virus X protein (HBx) has many cellular functions and it's the major factor of hepatitis and hepatocellular carcinoma caused by HBV infection. A proteomics approach was used for searching HBx interacting proteins, which may provide some new clues to molecular mechanisms of hepatocarcinogenesis. Retroviruses vector contained HBx gene and TAP tag was packaged and used to infect HepG2.2.15 cells. Then the stable cell line expressing TAP tagged-HBx protein was selected by resistant screening. For investigation of HBx interacted proteins, those infected cells were lysed and the protein complexes contained HBx were purified by Tandem Affinity Purification coupled with Mass Spectrum (TAP/MS). Finally we obtained about 60 protein complex components. Besides four reported interacted proteins of HBx (HSPA1A, DDB1, TFIIB and HSPD1), most were newly identified as HBx interaction candidates in our study. The dataset covered some functional categories such as cell apoptosis, transcription regulation and signaling transduction pathway which have been reported to be related to HBx functions. Further analysis revealed that those proteins are mainly localized in cytoplasm which coincided with the dynamic localization of HBx in cells reported before.

PP-348

Predictors of significant histological findings in chronic hepatitis B patients with persistently normal ALT levels

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Aim: To find the predictors of significant histological findings (HAI≥4 and/or Ishak≥3) in CHB patients with persistently normal ALT levels (PNAL).

Methods: 139 patients who had PNAL (defined as normal ALT measured on at least 3 occasions in the intervals of more than two months over a period of ≥12 months apart prior to biopsy) were underwent percutaneous liver biopsy.

Results: Even though 66(47.5%) patients with PNAL had normal liver histology, 33(23.7%) were found to have significant histological findings, even 13(9.4%) had established cirrhosis. When compared to patients within 0–0.75×ULN ALT, patients within 0.75–1×ULN ALT had higher rate of significant histological findings(p=0.029). In the subgroup, significant histological findings increased sharply after the age of 40 yrs as seen in other cohorts. However, significant histological findings were not identified by viral load or HBeAg status.

Conclusions: Liver biopsy should be considered in CHB patients with PNAL and detectable viral load, regardless of HBeAg status or viral load levels, especially in those older than age 40 yrs and higher ALT within 0.75–1×ULN.

PP-349

A1762T and G1764A mutations of hepatitis B virus, associated with increased risk of hepatocellular carcinoma, reduce the enhancer II/basal core promoter activity

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Background: Recent studies reported that basal core promoter mutation (A1762T and G1764A) was associated with more aggressive progression of liver disease from inactive carrier to active hepatitis, and eventually to liver cirrhosis and HCC. But the effect of the double mutations on the activity of enhancer II/basal core promoter is still uncertain.

Objectives: To evaluate the influence of nt1762 A/T and nt1764 G/A mutations on HBV enhancer II/basal core promoter activity.

Methods: The PCR fragments of HBV enhancer II/basal core promoter (nt1601 to nt1815) from the serum-derived genotype B HBV DNAs of one HBV carrier aged 66 and one HBV related hepatocellular carcinoma patient aged 26 were introduced into the pGL3-Basic-Vector from Promega via restriction sites of Xho I and Hind III. The nt1762 A to T and T to A, the nt1764 G to A and A to G mutations were carried out by GeneTailor Site-Directed Mutagenesis System from Invitrogen. The promoter activity was evaluated by comparing firefly luciferase measurement with Renilla luciferase as the internal control using the Dual-Luciferase Reporter Assay System from Promega.

Results: The luciferase reporter assay results indicated that the 1762 T to A combined with 1764 A to G mutations increase ($P<0.001$) while the 1762 A to T combined with 1764 G to A mutations decrease ($P<0.05$) the HBV enhancer II/basal core promoter activity significantly.

Conclusions: Associated with increased risk of hepatocellular carcinoma, A1762T and G1764A double mutations of hepatitis B virus reduce the enhancer II/basal core promoter activity.

PP-350

Significance of hepatic steatosis in chronic hepatitis B infection

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Introduction: Hepatic steatosis has been found to be a common and important histological finding in chronic hepatitis C virus (HCV) infection. The impact or effect of steatosis or NAFLD on chronic hepatitis B virus (HBV) infection remains to be elucidated.

Aim: To determine the significance of steatosis on liver fibrosis in chronic HBV infection.

Methods: We studied 51 consecutive hepatitis B e antigen (HBeAg) positive chronic HBV patients with HBV DNA > 5 log10 copies/ml without steatosis (Group 1), and 61 consecutive HBeAg positive chronic HBV patients with HBV DNA > 5 log10 copies/ml with steatosis (Group 2) undergoing staging liver biopsy. Subjects with fibrosis stage 4 or above was defined as severe liver fibrosis and chronic hepatitis as modified histologic activity index (HAI) 4-16.

Results: Group 1 had a lower body mass index [median (range) 25 (18-32) vs. 28 (21-33) kg/m2, $p<0.001$] and was older [44 (22-65) vs. 38 (17-66) years, p=0.01] when compared with Group 2. Fibrosis stage was similar between the 2 groups [3 (0-6) vs. 3 (0-6), p=NS]. However, Group 1 was more likely to have chronic hepatitis on liver biopsy [38/51 (74.5%) vs. 11/61 (18.0%), $p<0.001$]. There was a trend that steatosis correlated with HBsAg staining (r=-0.17, p=0.08) but not with HBcAg staining (p=NS). Steatosis did not correlate with modified HAI or fibrosis stage (all p=NS). Modified HAI correlated with fibrosis (r=0.49, $p<0.001$).

Conclusion: Chronic HBV subjects with steatosis were more likely to have mild chronic hepatitis. Steatosis in chronic HBV infection is associated with higher BMI and younger age.

PP-351

SiRNA targeting HBV C gene inhibits hepatitis B virus expression and replication in BHK-21 cells

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To determine whether there is an inhibitory efficacy of RNAi induced by siRNA targeting HBV gene, we have studied the inhibitory effect of

C-specific siRNAs on HBV expression and replication.The HBVC genes were cloned to generate the vector pEGFP-C. Two C-specific siRNA-S1 and S2 with homologous in sequence to the AY517488 C gene were designed and synthesized, according to the HBV genome of CHB patients from 25 ethnic minorities in Yunnan province[GenBank accession numbers AY517488,AY517489]. Also, one nonspecific siRNA-S3 was designed randomly for negative control. They were cloned into vector pU6 to constitute siRNA-expressing vectors, which were then cotransfected into BHK-21 cells with target gene vector of pEGFP-C.It was found by microscopic fluorescence detection conducted after cotransfection at intervals of 12, 24, and 48h that the expression of EGFP of groups S1, S2 and S1+S2 were reduced significantly in BHK-21 cells at the 12h, in comparison with negative control group1and 2 ($P<0.01$). The results showed that transfection of siRNA-expressing vectors S1and S2 caused an 80% to 90% reduction in the expression of HBV C in BHK-21 cells, and the anti-HBV effects extended to 6 days cotransfection. The siRNA-S3 did not has the same effect.These results demonstrated that the replication and expression of HBV mRNA were inhibited significantly in BHK-21 cells transfected with S1, S2, or both, as compared with negative control groups ($P<0.01$). The results of sequencing and RT-PCR prove the same effects further.For the first time it has been found that RNAi induced by siRNA targeting HBV C gene is continuous and stable inhibition of HBV expression and replication in BHK-21 cells, our data suggest that RNAi may provide a viable therapeutic approach to treat HBV infection.

PP-352

Liver disease progression in chronic hepatitis B infected persons with normal serum alanine aminotransferase level: Update from the R.E.V.E.A.L.-HBV Study

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Background: Data on disease state transition and its risk predictors for chronic hepatitis B (CHB) virus infection with persistently normal serum alanine aminotransferase (ALT) levels are lacking.

Methods: A subset of the R.E.V.E.A.L.-HBV cohort free from liver cirrhosis and hepatocellular carcinoma (HCC) and with persistently normal serum ALT levels through the first year of enrollment was included. HBeAg, serum HBV DNA, and HBV genotype were tested on baseline serum samples. Cases of chronic hepatitis (CH), liver cirrhosis (LC), HCC, and liver-related death (LD) were ascertained through follow-up serum ALT levels, abdominal ultrasonography, and data-linkage with the computerized National Cancer Registry and Death Certification profiles. The sequential disease progression rates were estimated. In the Cox regression models, multivariate-adjusted hazard ratios for baseline characteristics and time-dependent intermediate disease states were derived.

Results: 2,097 subjects contributed 27,282 person-years of follow-up (mean, 12.5 years). New cases of CH, LC, HCC, and LD were 292, 109, 44, and 28, respectively. For HBeAg-seropositive active carriers (HBV DNA $\geq 10^4$ copies/mL), the sequential incidence rates (per 100,000 person-years) from the asymptomatic carrier state through CH, LC, HCC and LD were 3,089, 3,265, 3,005, and 24,933. Comparable figures for HBeAg-seronegative carriers with HBV DNA $\geq 10^4$ ($<10^4$) copies/mL were 2,017 (842), 2,728 (912), 3,928 (2,826), and 14,548 (5,932). Elevated serum HBV DNA levels increased the risk of disease progression to every advanced disease state.

Conclusions: Subjects with HBeAg-positive CHB had higher rates of disease state transition. Elevated serum HBV DNA level significantly predicted risk of disease progression in these subjects.

PP-353

HBV viral load less than 104 copies/mL is associated with significant risk of hepatocellular carcinoma in chronic hepatitis B patients: An update from the R.E.V.E.A.L.-HBV study

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Introduction: International treatment guidelines classify CHB patients with low level viremia ($<10^4$ copies/mL) as having inactive disease with little or no risk of disease progression. Risk of disease progression in these subjects has not been properly evaluated.

Methods: HBsAg(-) subjects without HCV infection and a subset of the R.E.V.E.A.L.-HBV cohort without HCV infection or liver cirrhosis at baseline were included in this analysis. All HCC cases were confirmed using established criteria. Multivariable adjusted hazards ratios (HR_{adj}), using time-dependent Cox proportional hazard modeling which included baseline characteristics (age, gender, habits of alcohol consumption and cigarette smoking) and HBV-DNA and ALT levels over time, were used to determine risk of HCC progression.

Results: A total of 18,541 HBsAg(-) and 3,584 HBsAg(+) subjects were analysed for a total 267,809.1 person years of observation over 12.1 years, and a total of 184 HCC cases. Compared with HBsAg(-) persons, the HR_{adj} (95% CI) of developing HCC by HBV-DNA level in HBsAg(+) persons was 3.0 (1.4–6.3) for undetectable; 3.3 (1.7–6.6) for 300–9,999; 14.4 (8.5–24.4) for 10,000–99,999; 32.0 (19.9–51.6) for 100,000–999,999; and 30.5 (20.1–46.2) for ≥1 million copies/mL after adjustment for baseline and follow-up characteristics.

Conclusion: Results show that CHB patients with HBV DNA viral load $<10^4$ copies/mL are at a significantly higher risk of developing HCC than HBsAg(-) persons. The risk of HCC associated with detectable HBV DNA level was 3-fold greater. During follow-up, HBV viremia between 300–9,999 copies/mL was associated with a significantly higher risk of HCC when compared to those achieving HBV DNA <300 copies/mL.

PP-354

High viral load predisposes ALT flares accompanying phenotypic virologic resistance to adefovir therapy in patients with lamivudine-resistant chronic hepatitis B

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Backgrounds/Aim: In this study, we intended to determine the predisposing factors of alanine aminotransferase (ALT) flares accompanying phenotypic viral resistance (PVR) to adefovir dipivoxil (ADV) therapy in patients with lamivudine (LMV)-resistant chronic hepatitis B (CHB).

Methods: Out of the 96 patients with LMV-resistant CHB who were treated with ADV at a dose of 10mg/day for a median 13 months (12–28), 22 patients (23%) experiencing PVR were subjected. PVR was defined as the increase of serum HBV-DNA level determined by real-time PCR more than 1 $\log_{10}$copies/mL at 2 or more consecutive tests. Serum ALT and HBV-DNA levels were measured at 2-3 month interval during the follow-up periods.

Results: The median level of serum HBV-DNA was 5.36 $\log_{10}$copies/mL (3.62-7.82) at the time of PVR. Out of 22 patients with PVR, 10 (45%) were accompanied by serum ALT flares at a median time of 3 months (0-5) after PVR. The median serum ALT level of patients with elevated values was 85 IU/L (55-282) at the time of ALT flare. Nine (69%) of 13 patients with serum HBV-DNA ≥ 5 $\log_{10}$copies/mL and only one (11%) of 9 patients with serum HBV-DNA < 5 $\log_{10}$copies/mL showed ALT flares following PVR ($P < 0.05$). In patients with ALT flare following PVR to ADV therapy, however, there was no correlation between the serum ALT and the HBV-DNA levels.

Conclusion: It is suggested that mainly the PVRs associated with high viral load may be accompanied by serum ALT flares during long-term ADV therapy in patients with LMV-resistant CHB.

PP-355

A real-time PCR assay for rapid detection of YMDD mutants and simultaneous quantification of hHepatitis B virus viral load

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Aim: To establish a rapid real-time PCR assay detecting tyrosine-methionine-aspartate-aspartate (YMDD) mutants and simultaneous quantification of hepatitis B virus (HBV) viral load.

Methods: We designed a common pair of forward primer and reverse primer to a highly conserved sequence within the polymerase open reading frame encoding the C domain of the viral enzyme. The total viral load was detected and quantified by using real-time PCR and a fluorescent probe 1 that annealed to a highly conserved region between the forward and reverse primers. Another fluorescent probe 2 targeting at YMDD motif was used to for discrimination between the wild type and YMDD mutants of HBV strains. Serum samples from 109 lamivudine-treated patients with chronic hepatitis B virus infection were detected using this method and the results were confirmed by DNA sequencing.

Results: 109 serum samples from HBV-infected patients with YMDD mutation all had only one fluorescent signal, no matter rtM204V/I combinatorial with rtL180M or not, which demonstrated the excellent concordance between sequence analysis and the real-time assay. The lower detection limit was 100 copies/mL.

Conclusion: This real-time PCR assay might provide a potential application to rapidly monitor YMDD mutants and simultaneously measure HBV viral load in the clinical setting.

PP-356

An association study between 15 polymorphisms in interferon pathway genes and response to interferon α treatment in hepatitis B patients

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Backgrounds: Interferon α (IFN α) therapy remains a mainstay of treatment in hepatitis B. In this present study, nested case control study was used to perform the association study between 15 polymorphisms in interferon pathway genes and response to IFN α treatment.

Methods: In 246 chronic hepatitis B patients treated by IFN α, 15 polymorphisms as IFNAR1 rs2252930, IFNAR2 rs2284551, rs2248202, rs4986956, OAS rs3177979, rs10849829, ADAR rs3738032, rs1127314, rs3766924, JAK1 rs17127090 and MxA G-88T, C-123A, C20A, rs467558, and rs469390 were detected by PCR-RFLP.

Results: After 3-6 months therapy, the total response rate is 68.7% (169/246). ALT values of baseline in response group are significantly higher than that in non-response group (171.2IU/L vs 100.3IU/L, P<0.001). Frequency of MxA20AA genotype in response group was significantly lower than that in non-response group (11.2%vs22.1%, P=0.026, OR=2.24, 95%CI=1.03-4.87). There were no clear differences in the frequencies of other 14 polymorphims between response group and non-response group.

Conclusions: High baseline ALT level is related to response to IFN α. Individuals carrying MxA20AA genotype are probably susceptible to non-response to IFN α.

PP-357

Hepatitis B in Australia: Results of the first National Hepatitis B Needs Assessment

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Hepatitis B is a global public health challenge. In Australia, 160,000 people are chronically infected and rates of hepatitis B-related liver cancer are increasing. Only 2% of people with chronic hepatitis B in Australia access antiviral therapy. Chronic hepatitis B infection often occurs within a highly disrupted social context and many communities most affected by chronic hepatitis B have limited access to health care services.

The National Hepatitis B Needs Assessment is the first exploration of the needs of people with hepatitis B and clinicians involved in their care. Data was collected from all states and territories through semi-structured interviews with people with hepatitis B (n=20), clinicians (n=30), government bureaucrats (n=15) and community workers (n=25), a questionnaire for General Practitioners (GPs) (n=95) and focus group discussions with community workers (n=40).

People with hepatitis B often reported their diagnosis as shocking. Many received inadequate information at diagnosis which led to confusion about managing their infection. Few resources were available and many attached a low priority to hepatitis B and relied on untrustworthy sources of information.

To date the Australian government response to chronic hepatitis B has been limited. Workforce development was identified as necessary for all professional sectors. The report study highlights the need for strengthening vaccination programs, increasing access to treatment, developing testing protocols, enhancing research activities and implementing workforce development programs.

PP-358

Visulize core protein of hepatitis B virus in living cell by biarsenical labeling

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In this study, a recently developed biarsenical-binding technique was applied to fluorescently label hepatitis B virus (HBV) core protein in living cells. This approach uses a relatively small tetracysteine tag that is genetically engineered into the protein of interest. This tag, minimally two pairs of cysteines held in a hairpin configuration, specifically reacts with membrane-permeable biarsenical compounds that selectively fluoresce when covalently bound to the cysteine pairs. Since this genetic tag is relatively small and simple, it can be placed into target proteins with minimal disruption to the protein. It has been reported that a chimeric HBV core protein of which GFP inserted the immunodominant c/e1 epitope site, could form capsid-like fluorescent particles. Therefore, we constructed variants of a virus replication competent plasmid containing a 1.3 times genome-length HBV. Each of variants encodes a chimeric core protein of which a tetracysteine tag is fused into the c/e1 epitope site. We dynamically observed HBV core protein expression, traffic and assembly in living cells using biarsenical compounds labeling a tetracysteine tag, which was inserted in the c/e1 epitope site of core protein. Moreover, it is shows that these core-TC chimeras can form capsid-like particles. These results demonstrate the biarsenical labeling technique is suitable to study HBV core protein expression, traffic and assembly in living cells. We can understand HBV assembly and biology utilizing the labeling approach.

PP-359

Progress of the development of a Chinese marmot-model for infection with woodchuck hepatitis virus

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The woodchuck (Marmota monax) model is an informative mammalian model for investigation of hepatitis B virus infection. Recently, we established a new model with a related Marmota species in China. It could be shown that a Chinese Marmota-species is susceptible to the experimental WHV infection. We addressed the further questions about this new model: 1. is it possible to further enhance the susceptibility of Chinese marmots by immunosuppression? 2. What is the immunogenetic relationship between the Marmota-species?14 animals were infected with WHV. Ten of the infected animals received a treatment with cyclosporine for four weeks while 4 animals served as control. All 10 animals developed acute WHV infected with viremia. Only 2 of 4 control animals were viremic. These results indicated that the immunosuppression did facilitate the establish acute WHV infection in Chinese marmots. A series of the genes of M. hymalayana and M. baibacina including the interferon-α gene family were characterized by cloning and sequencing. The characterized genes so far revealed a extremely close evolutionary relationship between the Marmota-species, as a homology over 99% was found for many genes like interleukin 6 and 10 from these species. This fact facilitates the use of Chinese marmots as the developed assays and reagents for the woodchuck model are directly transferred for the new model.

PP-360

Characteristics of chronic hepatitis B patients achieved HBsAg seroconversion during treatment with peginterferon alpha-2a

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Background and objectives: To understand the characteristics of CHB patients who achieved HBsAg seroconversion after peginterferon alpha-2a treatment in China , 62 patients were abserved

Methods: Patients were retrospectively divided into 3 groups according to the time of HBsAg responses: ≤24 weeks (early response), 24－48 weeks (late response), and >48 weeks (delayed response). Related predict factors were evaluated by Kaplan-Meier survival analysis and Log rank test based on gender, age, baseline ALT level, baseline HBeAg, and baseline HBV DNA level, the portions of patients in different groups were calculated.

Results: 50.1% of the cases achieved early response, 33.5% achieved late response and 16.1% achieved delayed response. In the early responders, 58% of the cases had baseline ALT level > 5 ULN, 45.1% had baseline HBV DNA $>10^7$ copies/ml, 35.5% had baseline HBV DNA within 10^5-10^7copies/ml. In the late response, 38% had baseline ALT level > 5 ULN, 47.1% had baseline HBV DNA $>10^7$ copies/ml, 47.6% had baseline HBV DNA within 10^5-10^7copies/ml. In delayed responders, 20% had baseline ALT level > 5 ULN, 40% had baseline HBV DNA $>10^7$ copies/ml. HBV DNA level in all three groups decreased to less than 10^3 copies/ml during first 24 weeks of treatment, but fluctuated within the range of 10^3-10^5 copies/ml in some responders.

Conclusion: Most of the patients with HBsAg seroconversion has high ALT and middle to high HBV DNA level before treatment. HBV DNA is not an ideal measurement for predicting HBsAg sero-conversion in late and delayed responders.

PP-361

Th17 expression in chronic hepatitis B (CHB) patients

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Background: Chronic Hepatitis B (CHB) is a hepatitis B virus (HBV) induced chronic disease which affects millions of people's life in the world, most of whom reside in Asia-Pacific region, such as Southern China and Taiwan. IL-17 secreted CD4 positive T cells (Th17) were related to many autoimmune system and chronic diseases and Th17's role in CHB is still not understood. We will study the expression of TH17 in CHB patients in this study.

Methods and subjects: Serum and periphery full blood were got from twenty CHB patients. Patients' serum HBV DNA was tested with quantity PCR and peripheral blood mononuclear cells (PBMC) were separated from heparin full blood with Lymphoprep (Nyegaard, Norway). Then PBMC were stained with antibodies: ECD- anti-hCD3, PC7- anti-hCD4 and PE- anti-hIL-17A through intercellular staining. Finally, stained PBMC were analyzed with flow cytometry (Beckman Coulter).

Patients were divided into two groups: HBV DNA low group (n=7) represented as serum HBV DNA level <10000 copies/ml and HBV DNA high group (n=13) represented as serum HBV DNA level >=10000 copies/ml.

Results: The percentage of TH17 in HBV DNA high group is 2.75+_0.74% and in HBV DNA low group is 1.76+_0.31%, and P value of them is 0.025.

Conclusion: High HBV DNA CHB patients express higher level of Th17 in periphery blood than low HBV DNA CHB patients.

Acknowledge: This research is supported by 973 program (2007CB512800) and RPG (HKU7679/06M) to Prof G. K. K. Lau and CSY foundation.

PP-362

A double- spliced defective hepatitis B virus genome derived from hepatocellular carcinoma tissue enhanced replication of full-length virus

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In Hepatitis B virus (HBV) replication cycle, pregenomic RNA undergoes splicing and the reverse transcribed defective genomes can be packaged and released. Various types of spliced defective HBV genomes have been isolated from the sera and liver tissues of viral hepatitis B patients. To explore the functions of a 2.2kb double spliced HBV variant, we amplified and cloned a 3.2 Kb full-length HBV isolate (#97-34) and its 2.2 Kb double-spliced HBV variant (#AP12) from the tumor tissue of a patient with hepatocellular carcinoma (HCC). Sequencing showed that #AP12 had deletions in pre-S2, part of pre-S1, S genes, part of the spacer, and part of the reverse transcriptase gene, while the X gene was intact. When his defective double-spliced genome and its full-length counterpart genome were co-transfected into HepG2 cells, the former was shown to enhance the replication of the latter, both by real-time PCR and southern blotting. The enhancing competency of #AP12 was shown to require an intact HBV Xexpression cassette. However, the magnitude of enhancement of #AP12 was approximately 10 times higher than that of the full-length genome. The double-spliced defective variant could contribute to persistent HBV replication in HCC patients.

PP-363

Sustained durability of HBeAg seroconversion in patients with chronic hepatitis B treated with telbivudine or lamivudine

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Background: Durable suppression of HBV is a key objective of CHB treatment. Discontinuation of therapy may be considered for HBeAg-positive patients who achieve HBeAg seroconversion. This analysis explores the off-treatment durability of response in HBeAg-positive CHB patients after discontinuation of telbivudine or lamivudine.

Methods: Data were pooled from 1,211 HBeAg-positive patients treated with telbivudine (600 mg/day) or lamivudine (100 mg/day) in two phase III studies. Patients who had received antiviral therapy for ≥1 year and had HBV DNA <5 log10 copies/mL, with HBeAg loss maintained on treatment for ≥24 weeks, qualified for treatment discontinuation. Regular follow-up continued for up to 52 weeks post-treatment.

Results: Compared with lamivudine, a higher percentage of telbivudine recipients qualified and discontinued due to efficacy; had confirmed seroconversion by discontinuation; and sustained seroconversion after 12, 24, and 52 weeks of off-treatment follow-up (Table). Among patients who discontinued due to efficacy and had seroconversion by treatment cessation, the Kaplan-Meier estimates of sustained off-treatment seroconversion rates were 98%, 94%, and 86% after 12, 24, and 52 weeks for telbivudine, and 100%, 95%, and 93% for lamivudine, respectively.

Conclusions: Post-treatment durability of seroconversion is similar in patients treated with telbivudine or lamivudine. However, higher proportions of HBeAg-positive telbivudine recipients achieve seroconversion and qualify for treatment discontinuation.

PP-364

Clevudine therapy in patients with HBV associated liver cirrhosis with rapid virological response

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Background: In the pivotal phase III clinical trials, clevudine 30 mg for 6 months showed potent antiviral activity and significant biochemical improvement along with a marked post-treatment antiviral effect. This analysis was performed to evaluated the early biochemical and virological response of clevudine in chronic hepatitis B patients with cirrhosis.

Methods: The data of 13 patients who were diagnosed chronic hepatitis B patients with cirrhosis were collected from 1 hospital. Preliminary results who have been treated for at least 1 month are presented here. HBV DNA was quantified by bDNA assay with a lower limit of detection of 141,500 copies/mL.

Results: Median HBV DNA levels before therapy was 7.3 $\log_{10}$ copies/mL and the median changes in HBV DNA levels from baseline was -1.5$\log_{10}$ copies/mL after 1 month of therapy (n=13) and -2.7 $\log_{10}$ copies/mL after 3 months therapy (n=4). Serum HBV DNA levels were below 141,500 copies/mL in 46% (6/13) at 1 month and 75% (3/4) after 3 months of therapy. At baseline, overall median ALT was 51 IU/L and 3 patients had normal ALT. Forty-six percent (46%, 6/13) at month 1 and 100% (4/4) of patients at month 3 had normal ALT.

Conclusion: Clevudine 30 mg once daily therapy demonstrated early viral suppression and significant biochemical improvement in patients with liver cirrhosis.

PP-365

Using pSIREN-RetroQ vector to inhibit the replication of hepatitis B virus by RNA interference in vitro

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Aim: Present therapy for chronic hepatitis B, including IFN-α and nucleoside analogue, achieves control of HBV infection only in limited proportion of patients. RNA interference (RNAi) is the process of sequence-specific gene silencing, initiated by double-stranded RNA that is homologous in sequence to the target gene. We tried to inhibit the replication of HBV by a reverse transcription virus vector which can express short hairpin RNA *in vitro*.

Methods: We constructed pSIREN vectors with inserted oligonucleotides targeting on RT regions of HBV genome. These plasmids were co-transfected with pHBV3.8 into Huh-7 cells. Viral antigens were measured by ELISA. HBV core particle DNA were measured and quantified by Real-time PCR and Southern blot. Viral RNA was analyzed by Northern blot.

Results: We found that vector-based siRNA could potently reduce hepatitis B virus antigen expression in transient replicative cell culture. One of our constructed plasmids (312i) significantly decreased levels of viral proteins, RNA and DNA in cell culture. We found that the inhibition effect of RNAi is dose dependent and it can suppress pHBV3.8 expression at least for 144 hours. We also found that if pSIREN vectors were transfected earlier than pHBV3.8, silent effect may better than after pHBV3.8.

Conclusion: We established a new RNAi system that can inhibitor HBV expression and replication in human hepatocellular carcinoma cells. Theses findings indicated that RNA interference may be a potential tool to control HBV infection. RNAi-Ready pSIREN-RetroQ Vector may become a potent tool to inhibit HBV expression and replication.

PP-366

GTPase activity is not essential for the interferon-inducible MxA protein to inhibit the replication of hepatitis B virus

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Multiple studies have established that GTPase activity is critical for MxA to act against RNA viruses. Recently, It was shown that MxA can also restrict the replication of hepatitis B virus (HBV), a DNA virus, but the requirements for GTPase activity in its inhibition against HBV remain unknown. Here, we reported that GTPase defective mutants （K83A, T103A, and L612K）could block the secretion of the extracellullar HBsAg and HBeAg and reduce the expression of extra- and intracellular HBV DNA in HepG2 cells at levels similar to that by wild-type MxA. Importantly, extracellular HBeAg and HBsAg and extra- and intracellular HBV DNA in the GTPase defective MxA mutants (K83A, T103A, and L612K) groups displayed no marked difference in comparison to the wild-type MxA group($p > 0.05$). MxA protein was detected predominantly in the cytoplasm of uninfected cells; however, in HBV-expressing cells, MxA redistributed partly into nucleus. Furthermore, TMxA and T103, two nuclear forms of wild-type MxA and GTPase defective mutant (T103A), just could slightly downregulate the extracellular HBeAg, and decrease the expression of extra- and ntracellular HBV DNA in HepG2 cells. In conclusion, in contrast to the critical role of MxA GTPase activity in preventing the replication of RNA viruses, GTPase activity is not essential for MxA protein to inhibit HBV replication. This study also indicates that MxA protein may just have a minimal effect on the replicative cycle of HBV in the nucleus**.**

PP-367

High serum adiponectin correlates with advanced liver diseases in patients with chronic hepatitis B

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Adiponectin possesses anti-inflammatory and insulin-sensitizing properties. Little is known about the role of adiponectin in hepatitis B-related liver disease. Serum adiponectin and hepatitis B viral factors were cross-sectionally assayed in 280 patients with chronic hepatitis B virus (HBV) infection, including 120 chronic hepatitis B patients, 40 cirrhosis patients and 120 patients with hepatocellular carcinoma (HCC), and 116 healthy adults as controls. The dynamics of serum adiponectin level was also studied longitudinally in 25 patients with hepatitis B e antigen (HBeAg) seroconversion. We found that serum adiponectin level in chronic hepatitis B patients was similar to that in healthy controls, and was significantly lower than that in cirrhosis and HCC patients. In univariate analysis, high serum adiponectin level significantly correlated with the presence of HBV-related cirrhosis or HCC, abnormal serum ALT level, and HBV genotype C. After adjustment for age and gender, high serum adiponectin level was significantly correlated with the development of HCC (odds ratio: 5.56, 95% confidence interval: 2.94-10.52). Serum adiponectin levels remained stationary in patients experiencing HBeAg seroconversion. Our findings suggest that Serum adiponectin level correlates with the progression of HBV-related liver diseases, but not with the development of HBeAg seroconversion.

PP-368

Proteomic analysis of hepatitis B virus (HBV) response factors in an inducible system

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Hepatitis B virus (HBV) infection is one of the major threats to public health worldwide, which leads to chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). Although considerable progress has been made in the past years, the pathogenesis of HBV infection and the mechanisms of host-virus interactions are still elusive. In this study, we performed comparative proteomics to globally analyze the host response to HBV by using an inducible HBV-producing cell line. The combination of 2-DE and MALDI-TOF MS revealed that 16 proteins were up-regulated and 8 proteins were down-regulated by viral replication. The differentially expressed proteins can be functionally grouped into 6 categories: (1) protective enzymes against oxidative stress (PDHB, SOD1, ALDH2, PRDX2, PRDX3), (2) metabolic proteins (ATP5D, ATP5B, Hnrpk, FABP1, CTSD), (3) cytoskeleton and transport proteins (ACTG1, TUBA1B, LMNB1), (4) DNA replication, repair and transcription-associated proteins (PCNA, SNW1), (5) heat shock proteins and chaperones (HSPD1, GRP78, ERP60, CCT5, CCT6A), (6) cell growth, metastasis and signaling-associated proteins (RPSA, CRP55, ENO1, PHB). The results were further confirmed by real-time PCR and western blotting. Our studies will help us to further understand the pathogenesis of HBV and the host-virus interactions, and to develop biomarkers for diagnosis and targets for anti-HBV treatment in the future.

PP-369

The role of liver-targeted Foxp3+ regulatory T cells in the patients with chronic hepatitis B

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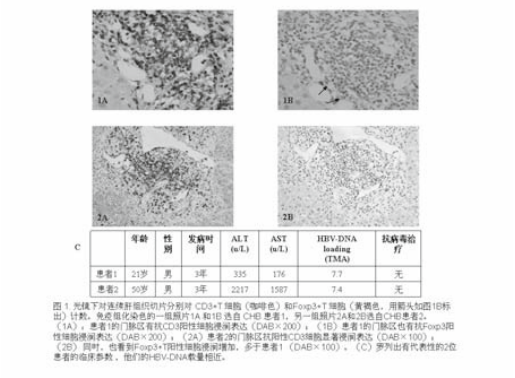
Objective: To study the correlation between the percentage of intrahepatic $Foxp3^{+}$Tregs and liver inflammation in chronic hepatitis B (CHB) patients, and address the role of regulatory T cells (Tregs) in the immunopathogenesis of chronic hepatitis B virus (HBV) infection.

Methods: 26 cases of CHB patients admitted to First Department of Internal Medicine, Nagasaki University School of Medicine, Sakamoto, Nagasaki, Japan were enrolled in this study. Clinical data including ALT、AST、HBV DNA level and inflammation score of liver pathology were collected. $CD3^{+}$T cell and $Foxp3^{+}$T cell in liver were detected by immunohistochemistry.

Result: CHB patients tended to have much more obvious liver damage in parenchymal area than that in portal area and reversely Foxp3+Tregs in serial sections accumulated mainly in the portal area. There was no significant correlation between the percentage Foxp3/CD3 and inflammatory activity of histological activity index score (HAI) were found in these patients ($p=0.116$). However there was a significance correlation between the percentage Foxp3/CD3 and liver Parenchyma inflammation ($p=0.0076$), moreoever Foxp3+Treg of high serum ALT level of CHB presented a higher frequency than those ones of low ALT level, and there was a significant difference between these two group. ($p=0.025$). Conclusions Our results suggest that Foxp3+Treg may play a major role in the pathogenesis of live cell injury in the CHB.



C

	年龄	性别	发病时间	ALT (u/L)	AST (u/L)	HBV-DNA loading (TMA)	抗病毒治疗
患者1	21岁	男	3年	335	176	7.7	无
患者2	50岁	男	3年	2217	1587	7.4	无

图 1 光镜下对连续肝组织切片分别对 CD3+T 细胞（咖啡色）和Foxp3+T 细胞（黄褐色，用箭头如图1B标出）计数。免疫组化染色的一组照片1A 和1B 选自 CHB 患者1，另一组照片2A和2B选自CHB患者2。（1A）：患者1的门脉区有较CD3阳性细胞浸润表达（DAB×200）；（1B）患者1的门脉区也有较Foxp3阳性细胞浸润表达（DAB×200）；（2A）患者2的门脉区较阳性CD3细胞显著浸润表达（DAB×100）；（2B）同时，也看到Foxp3+T阳性细胞浸润增加，多于患者1（DAB×100）。（C）罗列出有代表性的2位患者的临床参数，他们的HBV-DNA数量相近。

PP-370

The roles of tank-binding kinase-1 in chronic HBV infection induced interferon antiviral immunity

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Aims: To elucidate the roles of TBK1 on mDCs in hepatitis B virus (HBV) infection.

Methods: Peripheral blood monocytes were separated by combination of lymphocyte separating medium and CD14 magnetic microbeads from healthy volunteers (HV) and chronically HBV-infected patients (CH). mDCs were induced and proliferated in the culture medium with hGM-CSF and hIL-4. We stimulated mDCs with PolyI:C at the concentration of 25μg/ml. The mRNA expressions of TBK1, IRF3 and IFN-β were quantified by Real Time PCR. The levels of IFN-β in supernatant were determined by ELISA.

Results: The mRNA expressions of TBK1, IRF3 and IFN-β have no significant changes at 0, 12, 24 and 48h following the stimulation of PolyI:C in CH groups. However in HV groups there were significant elevation of IFN-β level at 12h following the stimulation of PolyI:C. The concentration of IFN-β has no significant changes at 0, 12, 24 and 48h in CH groups, whereas, it is a significant up-regulated at 12h in HV groups.

Conclusions: Our results suggest that abnormal expression of TBK1、IRF3、IFN-® may lead to persist infection of HBV.

PP-371

The effect of surface molecule expression level of dendritic cells on depressing HBVDNA of kushenin injection

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Aim: To test the effect of kushenin injection on the level of HBV-DNA and the surface molecules of dendritic cells in HBV transfenic mice.

Methods: 20 HBV transgenic mice were randomly divided into physiologic saline and Kushenin injection group. The normal group was 10 normal mice with same species and age. The mice in kushenin injection group were administrated at dosage of 82.2mg/kg·d -1by intraperitoneal and the mice in physiologic saline control group and normal group were administrated normal saline at the same volume for 30 days. The contents of HBV DNA in serum and liver were quantified by PCR. The surface molecules of dendritic cells were tested by flow cytometry.

Result: There was no significant difference of the serum HBV-DNA level between physiologic saline and Kushenin injection groups before management. After management, the content of serum HBV-DNA both showed a significant decrease. And the content of serum HBV-DNA in kushenin injection group dropped significantly when compared with the physiologic saline group. There was no siginificant difference in the content of HBVDNA in liver between physiologic saline and kushenin injection groups.The expression level of MHC-II significantly decrease in HBV transgenic mice than normal mice. Compared with physiologic saline group，the expression level of MHC-II in kushenin injection groups showed a significant increase.

Conclusion: KuShenin injection was effective on depressing HBV-DNA in HBV transgenic mice. Its antiviral action maybe achieved through regulating some of the molecular expression level in DC surface, especially MHC-II.

PP-372

The study of relativity PolyI:C triggering to secretion of type I interferon of dendritic cells' derive from peripheral blood monocyte of chronic hepatittis B patients

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Objectives: To investigate the secretion of type I interferon by peripheral blood dendritic cells (DCs) derived from peripheral blood monocytes of chronic hepatitis B patients when given polyriboinosinic:polyribocytidylic acid (PolyI:C) and to study the mechanism of DC's impaired function in chronic and persistent HBV infection accordingly.

Method: Ten chronic hepatitis B patients and ten healthy controls were randomly included in the study. The monocytes isolated from peripheral blood of candidates were incubated with rhGM-CSF and rhIL-4 to induce the DCs generation and proliferation. Then the morphotype of DCs was identified by microscope. At the sixth day of the incubation, PolyI:C, the ligand of TLR3, was treated. The interferon (IFN) production at different time-point after that was qualified by ELISA.

Result: 1 DCs in vitro can proliferate evidently when stimulated by cytokines with AIM-V.2 After PolyI:C triggering, the peak concentration of IFN-α in CHB patients group was similar to that in healthy controls group. 3 Compared with healthy controls group, DCs derived from CHB patients show less and weaker response to PolyI:C. 4 The plasma concentration of IFN-β of CHB patients was lower than normal people.

Conclusion: 1 AIM-V can meet the demands of in vitro culture of DCs.2 The secretion of IFN-α of DCs after PolyI:C triggering showed little difference on CHB patients or healthy controls. 3 The secretion of IFN-β of DCs from CHB patients after PolyI:C triggering decreased.4 The plasma concentration level of IFN-α in patients was higher than normal people, while the plasma concentration level of IFN-β was lower.

PP-373

Evolution of hepatitis B virus polymerase RT domain gene in the viral quasispecies during nucleos(t)ide analogues therapy

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Nucleos(t)ide analogues are a fundamental tool for treatment of chronic hepatitis B virus. However high resistance emergence, which is tightly related with the mutation of reverse transcriptase (RT) gene, is associated with the extension of treatment. We analyzed the genetic evolution of RT gene of viral quasispecies in a chronic hepatitis B patient who received lamivudine and adefovir therapy. Fifty colonies with HBV insert were selected for each sample. The rtM204V/L lamivudine-resistance mutation was already detected in 4.4% of clones prior to lamivudine therapy. At month 4.5 during lamivudine treatment, the rtM204I mutation became predominant, being present in 80% of clones. The rtM204I were associated with compensatory mutations (rtL180M, rtT184L). 9% of clones harbored the rtL180M+ rtT184L+ rtM204I mutations. In addition, two new compensatory mutations rtL229V and rtV191I were detected in 75% and 11.4% of clones, respectively. The patient was switched to adefovir therapy after lamivudine breakthrough. After 1 month of adefovir therapy, lamivudine-resistance mutations (rtL180M, rtT184L, rtV191I and rtM204I) disappeared. By month 18 of adefovir treatment, the rtN236T adefovir-resistance mutation was detected in 59% of clones. In addition, two new additional mutations rtA181V and rtM250L were found in 3% and 20.6% of clones, respectively. We also detected several polymorphic sites including rtT213S, rtF221Y, rtS223A, rtI224V, rtN238H, rtL267Q and rtQ271M .In conclusion, the risk of emerging drug-resistant mutants is a major problem of nucleos(t)ide analogues therapy. HBV quasispecies evoluted under the selective pressure of lamivudine and adefovir. The emergence of adefovir-resistant viral quasispecies was later than lamivudine-resistant strains during treatment.

PP-374

Proteomics study on an HBsAg stably expressing cell revealed decrease of a chaperon protein GRP78

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To study the possible effects of HBsAg on host cells, an HBsAg stably expressing HepG2 cell line (HepG2-S-G2) and its counterpart control cell line (HepG2-Neo-F4) were established. The differential protein expression profiles of HepG2-S-G2 and HepG2-Neo-F4 were compared using two dimensional gel based differential proteomic approach. Totally, 42 proteins were found decreased in HepG2-S-G2 cell compared to the control while 47 proteins were increased. All the proteins identified by MS/MS fell into several categories including metabolism-associated, immune-response-related, protein modification, signal transduction and others. Among them, GRP78/Bip, an important chaperone protein involved in multiple functions in host cells, was consistently decreased in HepG2-S-G2 and in Huh7 cell transfected with HBsAg expression plasmid. GRP78 belongs to the heat shock protein 70 family, which plays a crucial role in differentiation, proliferation and apoptosis pathways. Co-immunoprecipitation result showed direct interaction between HBsAg and GRP78. To further study the role of reduced GRP78 in cell functions, cell counting assay showed a decrease in cell proliferation. Cleaved PARP and release of cytochrome C from mitochondria were detected, which suggested apoptotic cell death. These results revealed a possible pathogenesis induced by HBsAg via GRP78.

PP-375

Correlations between viral loads in peripheral blood mononuclear cells and serum of chronic hepatitis B patients

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Background/Aims: There have been conflicting reports on the presence of HBV replicative intermediates in peripheral blood mononuclear cells (PBMC) and serum from patients with chronic hepatitis B (CHB) infection. We examined HBV cccDNA and pgRNA in the serum and PBMC, and investigated the effect of lamivudine on the viral loads in the PBMC of CHB patients.

Methods: In the crosssectional study, paired serum and PBMC samples from 50 treatment naive CHB patients were assayed for HBV total DNA, cccDNA and pgRNA. In the longitudinal study, 30 patients treated with lamivudine therapy were followed-up for a median of 34 weeks. And the viral loads were tested in the serum and PBMC samples.

Results: HBV cccDNA and pgRNA were undetectable in all serum samples, and HBV cccDNA was under the lower detection limit in most PBMC samples (84%). For all 50 patients, both the quantity of HBV DNA ($r=0.889$, $p<0.001$) and pgRNA ($r=0.696$, $p<0.001$) in PBMC correlated significantly with the quantity of HBV DNA in serum. In the longitudinal study, the reduction of both HBV DNA and pgRNA in PBMC were significantly lower than that of HBV DNA in serum ($P<0.05$).

Conclusions: (1)HBV cccDNA and pgRNA were undetectable in the serum of CHB patients; (2) HBV viral loads in PBMC were associated with the serum HBV DNA; (3) Lamivudine therapy had less effect on the HBV viral loads in PBMC compared with the serum viral loads. The inefficiency of antiviral therapy on PBMC viral loads may relate with the HBV persistence.

PP-376

Impaired TLR-IFNα signaling in plasmatytoid dendritic cells of chronic hepatitis B virus patients: possible role of HBsAg mediated suppression by BDCA-2 ligation.

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Although decreased frequency and function of circulating pDCs in CHB patients have been reported in previous research, the underlying mechanism of these defects remains unclear. In this study, we investigated whether HBV directly inhibits IFNs production in pDCs through HBV surface antigen (HBsAg) and whether this inhibition is associated with blocking of IFNs production-related signal pathway in cells. Intracellular cytokines stain assay showed that the ability of IFN-α secretion was remarkably impaired both in PBMCs and in pDCs from CHB patients when stimulated with TLR7 and TLR9 ligands. Isolated pDCs from healthy donors also exhibited suppressed IFN-α production when treated with HBsAg. This suppression was specific for TLR9, but not for TLR7. Besides, HBsAg inhibited TLR9 ligand-induced maturation of pDCs and proinflammatory cytokines production. Furthermore, TLR9-mediated IRF7 expression and nuclear translocation, which are important for activation of IFN-α gene transcription, were also blocked by HBsAg. Besides, inhibition of IFN secretion was associated with binding of HBsAg to BDCA2 which is one of the IFN-α production inhibitory receptors on pDCs. Taken together, these data suggested that HBV directly inhibited production of IFNs from pDCs through blocking of IFNs production-related signal pathway by HBsAg.

PP-377

Study on the immune responses induced by HBcAg and/or IL-18 gene modified dendritic cells in HBV transgenic mice

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Objective: To investigate the immune responses against HBV induced by the genes of HBcAg and/or IL-18 recombinant adenovirus-transduced dendritic cells in HBV transgenic mice.

Methods: DCs were generated from bone marrow cells from the HBV transgenic mice, and transfected with the genes of HBcAg and/or IL-18 recombinant adenovirus Ad-C-IL-18(DC/Ad-C-IL-18), Ad-C (DC/ Ad-C) and Ad-IL-18(DC/Ad-IL-18). HBV transgenic mice was received DC/Ad-C-IL-18, DC/ Ad-C or DC/Ad-IL-18 respectively at 3-weeks intervals. Intracellular cytokines of splenic T cells, HBcAg-specific activity of splenic CTL and anti-HBc and HBVDNA titers in sera were detected after immunization by flow cytometry, LDH release assay, ELISA and quantitative PCR, respectively.

Results: DC/Ad-C-IL-18, DC/ Ad-C, DC/Ad-IL-18 in turn stimulated efficient splenic Tc to secrete IFNγ and in turn induced stronge HBcAg-specific CTL response. The suppression of serum HBsAg and HBVDNA and the reduction of expression of HBcAg and HBsAg in liver in HBV transgenic mice induced by DC/Ad-C-IL-18, DC/ Ad-C or DC/Ad-IL-18 were in turn stronge.

Conclusion: HBcAg and/or IL-18 recombinant adenovirus-transduced DCs can in turn induced stronge HBcAg-specific Tc1 and CTL response, and reduced the titer of serum HBsAg and HBVDNA, and reduced the expression of HBcAg and HBsAg in liver in HBV transgenic mice, and especially DC/Ad-C-IL-18 maybe a promising candidate for the therapeutic vaccine for chronic HBV infection.

PP-378

MicroRNA expression profiles upon HBV infection

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MicroRNAs (miRNA) are associated with a variety of biological or pathological processes, including HBV infection. Viruses can not only encode miRNAs themselves but also affect host cells to express changed miRNA profile that will benefit the viral replication. In this study, we firstly investigated whether the miRNA expression profile was changed upon HBV infection, by using miRNA chip assay and followed by the confirmation of qRT-PCR assay. Results showed that there were six miRNAs (hsa-miR-30a-5p, hsa-miR-24, hsa-let-7a, hsa-let-7c, hsa-let-7f and hsa-miR-23b) down-regulated and three miRNAs (hsa-miR-194, hsa-miR-200a and hsa-miR-345) up-regulated in HepG2.2.15 cell line containing whole HBV genome. Furthermore, the changed miRNA profile was HBV specific, by comparison with other viruses. Finally, by using qRT-PCR assay, we found that miRNAs hsa-let-7a and hsa-let-7f were significantly down-regulated in liver samples from CHB patients. Therefore, these miRNAs might contribute to HBV infection and would be the targets of our future research.

PP-379

Symptomatic chronic hepatitis B (CHB) patients express higher level of IL-23p19 mRNA than asymptomatic CHB patients in Peripheral Blood Mononuclear Cells (PBMC)

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[1] *HKU*

Background: Chronic Hepatitis B (CHB) is a hepatitis B virus induced chronic disease which affects millions of people's life in the world, most of whom reside in Asia-Pacific region, such as Southern China and Taiwan. There are two statuses of CHB: symptomatic status

represented as high alanine aminotranferases (ALT) level (>=40 IU/L) and asymptomatic status represented as normal ALT level (<40 IU/L).Interleukin 23 (IL-23) is one member of Interleukin 6 (IL-6) family, which is constituted by two subunits: one is IL-12/IL-23p40 which is shared by Interleukin 12 (IL-12) and IL-23, the other is p19 which is the specific subunit of IL-23. IL-23 was reported to be the main regulator of Interleukin 17 (IL-17) and IL-17 was reported to be related to CHB, so IL-23p19 was studied in CHB.

Methods and Subjects: Serum and peripheral full blood were got from ninety four CHB patients. Serum ALT level was tested in Queen Mary Hospital Clinic Lab. Patients' peripheral blood mononuclear cells (PBMC) were separated from heparin full blood with Lymphoprep (Nyegaard, Norway), and the total RNA was isolated from PBMC with Qiagen RNA kit, and the mRNA level of IL-23p19 were semi quantified with Fam labeled human IL-23P19 primer and VIC labeled human GAPDH primer(ABI). The data were analyzed by SPSS software.

The ninety four CHB patients were divided into two groups according to ALT level (symptomatic group: ALT>=40 IU/Land asymptomatic group: ALT<40 IU/L):

Results: IL-23p19mRNA expression is higher in symptomatic status (1.67+_0.61) than in asymptomatic status (2.68+_0.51): P value is 0.013

Conclusion: Symptomatic CHB patients express higher level of IL-23p19 mRNA in PBMC than asymptomatic CHB patients.

PP-380

Two methods of immunomics used to identify a repertoire of autoantibodies in hepatitis B surface antigen positive transgenic mice

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Autoantibodies triggered in viral or non-viral hepatitis can often lead to a series of impairments of livers, which are prone to cirrhosis. High level of IgG was found in the sera of a number of chronic hepatitis B virus (HBV) and hepatitis C virus infected patients. In the sera of a lineage of hepatitis B surface antigen (HBsAg) expressing transgenic mice established in our lab, high level of IgG was detected using automatically analysis with Roche Modular P and western blotting. These IgGs were not anti-HBs confirmed by ELISA kit. For biomarker discovery, two approaches of discovery-driven immunomics were employed to identify the repertoire of autoantibodies in the sera of HBsAg expressing transgenic mice. One approach was Top-Down, in which the total proteins of mice livers were separated by 2DE, and then immunodetected using mice sera from HBsAg positive mice and negative control mice. The distinct spots were picked and identified by MS/MS. The other approach was Bottom-Up, in which the total liver proteins were separated by two sequential steps of affinity chromatography using the control mice sera and HBsAg positive mice sera respectively. Eluates from both steps were identified by MS/MS followed by results comparison. In virtue of two approaches of immunomics above, the repertoire of autoantibodies in the sera of HBsAg transgenic mice was obtained. The new putative biomarkers from HBsAg related autoantibodies can be identified by these methods.

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IL-17 stimulating HBV leaving from peripheral blood mononuclear cells may help HBV clearance in the serum of chronic hepatitis B patients under Pegy-Interferon treatment

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Background: Although previous study implied that IL-17 maybe helpful to inhibite viral infection, the effects on HBV remain unknown. We assessed IL-17 expression in the CD4+ T cells (Th17) of 8 consecutive CHB patients treated with pegylated interferon α-2a for 48 weeks and tried to explain the mechanism.

Methods: Th-17 were analyzed by flow cytometry on PBMCs of 8 CHB patients (4 with sustained virological response SVR; and 4 without SVR) at every 4 week during treatment until end of therapy (week 48) and every 12 week until the end of follow-up (week 72). HBV DNA was quantified by Q-PCR. PBMCs, AD38 celline culture with HBV core antigen and IL-17 respectively. IL-17, HBV-DNA were assayed in both cells and culture media.

Results: In parallel with decline in serum HBV DNA, we found an increase in IL-17 at the end of follow-up when compared with baseline [mean±standard error of mean (SEM) 2.46±0.37 vs. 1.44±0.23 % respectively, p=0.03]. IL-17 at the end of therapy was also higher than at baseline [mean±SEM 1.93±0.23 % respectively, p=0.05]. In vitro study we proved that the HBv core antigen stimulated IL-17 expression in PBMCs and the culture media. Incubated with IL-17, HBV DNA reduced in the PBMCs and increased in the culture media at first. Then as culture went on, HBV DNA reduced significantly compareing with that of non IL17 control. IL-17 has no effects on HBV-DNA level in AD38 cell line culture.

Conclusions: IL-17 has effects to stimulate HBV leaving from PBMCs, which might be of helpful to the HBV clearance in CHB patients.

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Viral Hepatitis

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Background/Aim: Chronic hepatitis B subjects with normal alanine aminotransaminase (ALT) are usually considered to have minimal inflammation on liver biopsy. However, there is evidence that the current upper limit of normal (ULN) for ALT is too high.

Aim: To compare the histological profile of those with ALT less than 0.6-times ULN with ALT between 0.6-1.0-times ULN.

Method: One-hundred-and-fifty-three HBeAg-positive Chinese subjects were evaluated; 48 (31.4%) had ALT less than 0.6-times ULN (Group 1) while 105 (68.6%) had ALT between 0.6-1.0-times ULN (Group 2).

Results: Group 2 were more likely to have chronic hepatitis with severe activity when compared with Group 1 (p=0.04). The median (range) fibrosis stage was lower in Group 1 when compared with Group 2 (p=0.01). Group 1 were more likely to have fibrosis stage 0 [28/48 (8.3%) vs. 42/105 (40.0%) respectively, p=.04] and less likely to have severe fibrosis [0/48 (0%) vs. 8/105 (7.6%) respectively, p=0.05] when compared with Group 2. HBV DNA was higher in Group 1 [median (range) 7.71 (7.03 to >8.93) vs. 7.49 (6.21 to >8.93) $\log_{10}$ copies/ml, p=0.01].

Conclusion: HBeAg-positive subjects with ALT less than 0.6-times ULN for 18 consecutive months have mild histological disease.

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Diseases phenotype analysis and DNA methylation difference detection in monozygotic twin pairs infected with HBV

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Background and aims: HBV transmission is often from mother to baby and in a familial aggregation pattern in china. Recent research shows that intimate contact between family members isn't major reason, but genetic factors. Our clinical research found that several monozygotic twin pairs remained discordant for HBV infection excluding virus influence. For monozygotic twins sharing common genes, we hypothesize that epigenetic susceptibility plays a key role in determining diseases phenotype. Since DNA methylation is one of major epigenetic modification type, we examined differences in genomic CpG island DNA methylation of monozygotic twin pairs discordant for HBV infection.

Methods: All 36 twin pairs were analyzed by STR microsatellite polymorphisms scanning for zygosity identification, diseases phenotyping, HBV genotyping and virus quasispecies complexity analysis. MCA-RDA (methylated CpG island amplification followed by representational difference analysis) methods were applied to isolate the differentially methylated genes. The target genes were sequenced and analyzed by bioinformatics methods. Methylation status of candidate genes was tested by bisulfite modification-methylation specific PCR method.

Results: Among 28 monozygotic twin pairs, 10 pairs were discordant and 5 pairs were concordant for HBV infection excluding virus influence. MCA-RDA analysis showed that 41 genes may associate with phenotype discordance, further analysis indicated that 9 genes may be potential candidate genes, such as genes of TJP3, NAPA, MVD and SMTN et al.

Conclusion: Differential methylation of genes might be involved in the phenotype discordance between monozygotic twin pairs for HBV infection.

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Low rate of adefovir resistance-related mutations in polymerase gene of hepatitis B virus in lamivudine-resistant chronic hepatitis B patients not treated with adefovir dipivoxil

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Background: Adefovir dipivoxil is used for the initial treatment of chronic hepatitis B or rescue treatment of lamivudine-resistant chronic hepatitis B, and exhibits excellent antiviral activity. However,the presence of resistance to adefovir dipivoxil was more frequently in lamivudine-resistant chronic hepatitis B patients than in lamivudine-naïve patients during adefovir dipivoxil monotherapy. But the rate of adefovir resistance related mutations is little known in lamivudine-resistant patients before adefovir dipivoxil treatment.

Methods: The existence of adefovir resistance-related mutations was examined in the sera of 240 lamivudine-resistant chronic hepatitis B patients with breakthrough hepatitis and 100 antiviral-naïve chronic hepatitis B patients. Both polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) and directly sequencing of PCR product were used to detect resistant viruses.

Results: rtA181T mutant was detected in only two of the lamivudine-resistant patients, while none in the antiviral-naïve chronic hepatitis B patients. There was no rtN236T mutant detected in the two groups.

Conclusion: Our results suggest that the rtA181T mutant virus were present in a few lamivudine-resistant chronic hepatitis B patients before they have been treated with adefovir dipivoxil, but the rtN236T mutant was not detected. The rate of adefovir resistance-related mutations in polymerase gene of hepatitis B virus was low in such lamivudine-resistant patients before adefovir dipivoxil treatment.

PP-385

Hepatocyte nuclear factor 4-alpha plays a critical role on HBV transcription and replication in vivo

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Objectives: Previous in vitro studies showed that hepatocyte nuclear factor 4-α (HNF4α) could support hepatitis B virus (HBV) replication in non-hepatocytes. The aim of this study is to investigate if HNF4α plays a critical role in regulating HBV transcription and replication in vivo.

Methods: A replication-competent HBV construct pHBV4.1 and two HNF4α specific short hairpin RNAs (shRNA) expression plasmids were co-injected into BALB/c mice tails by hydrodynamics procedure respectively. Mice were sacrificed 3 days post-injection. HNF4α antigen and HBcAg expression in the livers were detected by immunohistochemistry assays. Total RNA and HBV replication medium extracted from the livers were analyzed by Northern and Southern hybridizion respectively to evaluate the HBV transcription and replication level in vivo. Mice co-delivery with pHBV4.1 and a negative shRNA expression plasmid were used as the control.

Results: The HNF4α antigen expression in the livers of mice co-injected with pHBV4.1 and HNF4α shRNA1, decreased to a very low level compared with other groups. By the same time, the HBV transcripts and virus replication medium level in the livers of this group were decreased obviously and the expression of HBcAg in the liver decreased too, compared with other groups, while in these groups of mice the expression of HNF4α antigen in the liver did not have obviously changes.

Conclusions: These results showed that inhabit HNF4α expression may lead to suppression of HBV transcription and replication in the mice liver, which indicated that HNF4α plays a critical role in regulating HBV transcription and replication in vivo.

PP-386

Three years of continuous treatment with entecavir results in high proportions of Chinese nucleoside-naive patients with undetectable HBV DNA: Results from studies ETV-023 and -050

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Background/Aims: The aim of chronic hepatitis B treatment is sustained suppression of HBV replication. 180 entecavir-treated patients from Chinese study ETV-023 enrolled in a long-term rollover study ETV-050. We present efficacy and safety results in a cohort of patients from ETV studies -023 and -050 who received 3 years of continuous therapy with ETV.

Methods: The Chinese ETV-continuous cohort consists of 160 HBeAg(+)/(-) nucleoside-naïve ETV patients from ETV study -023 who (a) had a virologic non-response after completing 52 weeks of blinded dosing or (b) have not achieved consolidated response after 96 weeks of therapy or (c) had a virologic breakthrough during the second year of dosing and (d) enrolled into ETV-050 with a treatment gap ≤35 days. In ETV-050, patients were treated with 1 mg of ETV. Efficacy and safety were evaluated.

Results: After 3 years of therapy, among patients with available samples, 133/149 (89%) achieved the endpoint of HBV DNA <300 copies/mL; 129/150 (86%) achieved ALT ≤ ULN; 30/150 (20%) experienced loss of HBeAg; and 12/150 (8%) of patients experienced HBeAg seroconversion. By protocol design, most patients with a consolidated response were not included in this cohort. Therefore, numbers and proportions of patients with HBeAg loss and HBeAg seroconversion represent additional patients achieving these endpoints during treatment in ETV-050.

Conclusions: High proportions of nucleoside-naïve patients who received continuous treatment with ETV during 3 years had suppression of viral replication and ALT normalization. Safety profile was consistent with the previously reported experience.

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Association between polymorphisms of transporter associated with antigen processing (TAP) gene 2 and prognosis of hepatitis B virus infection

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To investigate the correlation between transporter associated with antigen processing gene and prognosis of hepatitis B virus infection,124 individuals infected with HBV and 113 healthy controls were genotyped for TAP2 by PCR-RFLP and amplification refractory mutation system PCR methods. The results showed a significant increase of the TAP2*0201 allele in the recovered group compared to the chronic HBV infected group（P<0.05）and chronic hepatitis B with liver cirrhosis group（P<0.05）.The frequency of genotype TAP2*0201 in the chronic hepatitis B group was significantly lower than those of healthy controls (p<0.05).The frequency of TAP2*0102 of the chronic hepatitis B group was significantly higher than the recovered group (p<0.05), which was also associated with the higher ALT level, HBeAg and HBV-DNA. There was a significant linkage disequilibrium (△= 0.026, p<0.05) between DR4 and TAP2*0101 alleles in the chronic hepatitis B with cirrhosis group, while DR4 itself showed positive association with recovery. These findings as the first report also suggest that TAP2*0101 genotype carriers especially 0101/0101 homozygotes are susceptible to chronic hepatitis B and are prone to the complications with cirrhosis, while TAP2*0201 is in favor of convalescence from hepatitis B virus infection.

Acknowledgements: This work was supported by NSFC (No. 30571650) , 973 Project (No:2006CB504304) .

PP-388

Hepatitis B virus genotypes, sub-genotypes, pre-core and basal core promoter mutations in two largest provinces, Punjab and Sind of Pakistan.

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HBV genotyping has been done in most countries, unfortunately in Pakistan HBV genotypic distribution is still unclear. So the main aim of present study is to determine the prevalent genotype and sub-genotype in two most populated provinces i.e., Punjab and Sind in Pakistan. Patients from 8 cities of both provinces were screened for the detection of HBV DNA and 236 HBV DNA positive samples were selected for genotyping by PCR-RFLP. The results were further confirmed with whole genome and partial genome sequencing. Genotype D was detected as the most prevalent (93.22%) genotype in both provinces, while genotype C was present in 5.93% and genotype A in 0.85% samples. Regarding sub-types D1 was present in 84%, D2 in 8% out of 25 whole genome sequenced samples. C2 subtype was detected in 58.33% of S gene sequenced samples while D1 in rest of the 41.67 % out of 24 samples sequenced for S gene. Sub-types D1 is the most dominant in D while C2 is genotype C. 17.43% of genotype D samples had 8 bps deletions in BCP region, while 1 have 15bp deletion in pre-S2 region. Other pre-core and BCP mutations were like T1915 (100%), A1679 (86.96%), T1762 (39.13%) and A1764 (30.43%) were also detected in genotype D samples. In conclusion, genotype D sub-type D1 is the most prevalent HBV strain in Punjab and Sind provinces of Pakistan and 8 bps deletion mutants and were rather common in HBV carriers.

PP-389

Detection of HBV drug-resistant mutations in 1711 patients with chronic hepatitis B

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Objective: To investigate HBV drug-resistant mutations against nucleos(t)ide analogues in patients with chronic hepatitis B.

Methods: Total of 1740 serum samples from 1711 patients with chronic hepatitis B were detected. HBV reverse transcriptase (RT) gene fragment was amplified by nested PCR, an in-house assay developed by us. Direct sequencing of PCR product was performed followed by analysis of various missense mutations at 13 sites associated with HBV drug resistance.

Results: Success rate of sequencing was more than 99% and 91.9% for $\gamma 10^4$ IU/ml and $<10^4$ IU/ml HBV DNA positive samples, respectively. 31.9% (555/1740) samples were mutation-positive. There were 59 substitutions of V173L, 294 of L180M, 41 of A181V, 75 of A181T, 6 of A181V/T, 7 of T184L, 2 of 184A, 3 of 202G, 201 of 4 M204V, 230 of M204I, 35 of M204V/I, 32 of V214A, 2 of Q215S, 2 of L217R, 61 of L229V, 19 of L229W, 10 of I233V, 51 of N236T, 2 of M250I, and 10 of M250L. In addition, several kinds of unusual substitutions at analyzed sites were observed. M204I and M204V were major lamivudine-resistant mutations. The former was often emerged alone while the latter was usually accompanied with L180M. Inconsistent with previous study out of China, A181T was frequently identified in adefovir-resistant patients in our detection. Entecavir resistance was all occurred in LAM-refractory patients, with substitutions at M250L or T184L in most cases.

Conclusions: Multiple-sites mutation detection is valuable for verifying HBV drug resistance and thus helpful for adopting reasonable antiviral therapy in clinic.

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Vaccination with a fusion DNA vaccine encoding hepatitis B surface antigen fused to the extracellular domain of CTLA4 enhances HBV-specific immune responses in mice

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DNA immunization has shown great potential in the prevention and treatment of hepatitis B virus (HBV) infection, but its potency is limited. Fusion of specific antigens to extracellular domain of cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4) represents a promising approach to increase the immunogenicity of DNA vaccines. In the present study, we evaluated this interesting approach for its enhancement on HBV-specific immune responses and its antiviral effects in HBV transgenic mice. A fusion plasmid encoding the extracellular domain of CTLA-4 linked with HBsAg was constructed. Vaccination with the CTLA4-fused DNA vaccine resulted in much higher level of anti-HBs antibody with about several hundreds-fold enhancement, and also increased the antigen-specific T cell proliferation as well as CTL response in BALB/c mice. Moreover, this fusion DNA vaccine enhanced both HBsAg-specific IgG2a (Th1) and IgG1 (Th2) antibody responses, and induced both HBsAg-specific IFN-γ and IL-4 releases from stimulated splenocytes. In HBV transgenic mice, vaccination with this fusion plasmid down-regulated the expression of HBsAg and HBV DNA replication by induction of higher anti-HBs antibody and HBsAg-specific CD8+ response. The enhanced antiviral immunity of the CTLA4-fused vaccine is dependent on the binding affinity of CTLA-4 to B7 molecule. Our results suggested that the CTLA4-fused DNA vaccine might be used as not only a prophylactic but also a therapeutic vaccine in HBV infection.

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Programmed death 1 upregulation is associated with higher liver inflammatory reaction in chronic hepatitis B patients in immune clearance phase

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Aim: During immune clearance phase, immune-mediated lysis of infected hepatocytes become active.To test feature of PD-1 expression on PBMC and the effect of PD-1 expression on viral load and biochemical feature of chronic hepatitis B (CHB) patients in immune clearance phase, we cross sectionally studied the PD-1 expression on total peripheral CD8 and CD4T cell.

Methods: Forty-five CHB with ALT level increase were studied. PD-1 expression on total peripheral CD8 and CD4 T cell were evaluated by using flow cytometry.

Results: In CHB in immune clearance phase the percentage of PD-1 positive of total peripheral CD8T and CD4T cells were elevated compared to health control. A similar pattern was also observed for the mean fluorescence intensity (MFI) of PD-1 expression on these CD8T and CD4T cells in blood. Both the percentage of PD-1 positive and the MFI of PD-1 expression were not correlated with viral load. In contrast to viral load both ALT level and total bilirubin were correlated with the MFI of PD-1 expression on total CD8T cells significantly.

Conclusion: The level of PD-1 expression per cell rather than the presence or absence of PD-1 plays important role in regulating the immune-host interaction in CHB in immune clearance phase. And the MFI of PD-1 was correlated with high inflammatory reaction rather than viral load.

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Entecavir (ETV) salvage therapy for chronic hepatitis B patients who fail to respond to, or relapse from, adefovir (ADV)

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Background/Aims: The primary objective of antiviral treatment for chronic hepatitis B (CHB) is sustained suppression of viral replication and liver disease remission. Many CHB patients receiving adefovir (ADV) fail to suppress viral load to undetectable levels and increasingly report ADV resistance. We describe the outcome of ETV treatment in patients who failed/relapsed after ADV therapy.

Methods: Patients from study ETV-079, a randomized, open-label study comparing the antiviral efficacy, safety and viral kinetics of ETV (0.5 mg) versus ADV (10 mg) daily in nucleoside-naïve HBeAg(+). CHB patients could enrol into rollover study ETV-901 (ETV 1 mg/day) after 48 weeks.

Results: Eight ADV-treated patients in ETV-079 had median HBV DNA levels of 6.7 $\log_{10}$ copies/mL after 52 weeks (median exposure). All 8 enrolled in ETV-901 (6 sub-optimal responders had no treatment gap; 2 relapsed after 105 and 128 days off ADV) and received ETV (median exposure: 28 weeks). At Week 12 of ETV treatment, mean HBV DNA reduction was 4.59 $\log_{10}$ copies/mL and 4/8 achieved HBV DNA levels <300 copies/mL. At Week 24, the mean reduction in HBV DNA was 5.17 $\log_{10}$ copies/mL (N=6); all 6 achieved viral load reductions to $<10^4$ copies/mL and 3 achieved HBV DNA <300 copies/mL. Genotypic resistance testing was not performed. No patients experienced virologic breakthrough on ETV.

Conclusions: The majority of patients who were suboptimal responders to, or relapsed from, ADV treatment in study ETV-079 experienced rapid HBV DNA reductions when switched to ETV (study ETV-901), and HBV DNA continued to decline with extended ETV treatment.

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Inhibition of HBsAG and HBV DNA secretion by HBIG Nanoparticles (HBIG-PBCA-NP) in vitro

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Background/Aims: To investigate the inhibition of HBsAg release and HBV replication by HBIG poly(butylcynao- acrylate) nanoparticles （HBIG-PBCA-NP） in vitro.

Methods: HepG 2.2.15 were cultured with DMEM in the absence or present of HBIG-PBCA-NP or HBIG. The supernatant at some days interval was collected for HBsAg quantitative detection by time-resolved immunofluorometric assay (IFMA) and HBV DNA quantitative detection by real-time fluorogenic quantitative PCR(RTFQ-PCR).

Results: There was no cytotoxicity to the HepG2.2.15 cells in HBIG or HBIG-PBCA-NP concentration from 0.01 IU/mL to 10.0IU/mL. HBsAg and HBV DNA in supernatants of HepG2.2.15 cultured with 0.1, 1.0 or 10.0 IU of HBIG/ml or HBIG-PBCA-NP was reduced significantly than that of HepG2.2.15 cultured without drugs after 3, 6, 9 days incubation, respectively($P<0.01$). But there are no difference between HBIG- PBCA-NP and HBIG . HBsAg and HBV DNA in supernatants of HepG2.2.15 with 0.1, 1.0 or 5.0 IU/ml of HBIG-PBCA-NP or HBIG was reduced significantly, after three days incubation, respectively, as compared to the control ($P<0.01$). The amount of HBsAg and HBV DNA secreted in supernatants was reduced persistently at 5th and 7th days, and rebound at 9th and 11th days after HBIG-PBCA-NP or HBIG removed, respectively($P<0.01$), but the level of HBsAg and HBV DNA was lower in the supernatants of the culture with HBIG- PBCA-NP than that of culture with HBIG on the day 9 to 11 after the drugs removed ($P<0.05$ or 0.01).

Conclusions: HBIG-PBCA-NP can inhibite HBsAg release and HBV replication in vitro more persistent than that of HBIG.

PP-394

Effects of chronic hepatitis virus B infection on mRNA and protein expression, and enzyme activity of human hepatic cytochrome P450 2C9

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Objective: To investigate infection of chronic Hepatitis B Virus (HBV) on expression and enzyme activity of cytochrome P450 2C9 subfamily, involved in metabolism of many clinically therapeutics by using human liver S9 fraction prepared from liver tissues donated by patients with HBV infections and their controls.

Methods: 20 volunteers (male 4 and female 16) including 10with chronic HBV infection donated liver tissues during their surgical operations under informed consents, aged from 28 to 35 years old. Liver S9 fractions were prepared using procedure of differential centrifugation.CYP2C9 genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays. The activity of CYP2C9, tobutamide 4-hydroxylation, was determined by High Performance Liquid Chromatography (HPLC).The expression of CYP2C9 protein and mRNA were determined by Western blotting and RT-PCR.

Results: All 20 subjects showed CYP2C9 wild-type allele (*1) and none of them did CYP2C9 (*2) and CYP2C9 (*3). The mean Vmax of CYP2C9 in the subjects with HBV infections (236.7±61.9 pmol/mg/min) was significant lower than those without HBV infections (335.4±83.9 pmol/mg/min) ($P=0.008$). Similarly, CYP2C9 protein and mRNA expressed significantly lower in HBV infected than non-infected controls ($P<0.05$). However there was no significant difference of Km values for tobutamide 4-hydroxylation between HBV infected (48.9±13.7 uM) and non-infected controls (43.8±14.4uM) ($P=0.428$).

Conclusions: The enzyme activity of CYP2C9 was tendered in the subjects with HBV infections with a possible mechanism that this virus down-regulates the expression of human hepatic CYP2C9 both in protein and mRNA level. Clinical significance is addressed that chronic HBV infected might be subject to drug-drug interaction by CYP2C9.

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Related factors of lamivudine-resistant mutation in HBeAg positive patients with chronic hepatitis B

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Background/aim: Lamivudine (LAM) is a nucleoside analogue widely used to treat chronic hepatitis B virus (HBV) patients. But the emergence of LAM-resistant virus greatly limits the efficacy of therapy and develops the liver injury. The aims of this study were to investigate the related factors LAM-resistant mutation in HBeAg positive chronic hepatitis B (CHB) patients.

Methods: Thirty-five patients carrying LAM-resistant HBV with HBeAg positive were enrolled in this study. All of them underwent percutaneous liver biopsy, histological findings (necroinflammation with a score of HAI 4 and/or fibrosis Ishak's staged) and had detectable viral load (Taqman Real Time PCR). Age, viral load, levels of alanine aminotransferase, the types of resistant mutation (INNO-LiPA) and HBV genotype was monitored.

Results: The median year of mutation found was 24months (6-72 months). 85.71% were HBV C genotype, and 14.29% were HBV B genotype. The mutation of rtL80I, L80V, G173L, L180M, M204V and M204I were detected. The emergence rates were 34.29%, 25.7%, 17.1%, 60%, 57.1%, 54.3% respectively. Patients with two (42.86%) or three (32.43%) mutation simultaneously were much more than that with one or four (11.4%) mutation ($P<0.05$). Twenty-two (62.9%) patients were found to have significant histological findings, even 5 (14.3%) had established cirrhosis. Only two patients had no histological finding. One of them had rtL80I and M204I mutation. The others had rtL80V, L180M and M204V mutation. The number of resistant mutation has no significant finding with histological finding ($P>0.05$).

Conclusions: The emergence rate of L180M, M204V and M204I were higher than that of rtL80I, L80V, G173L in HBeAg positive CHB patients with LAM-resistance. Most of the patients have two or three LAM-resistant mutation no matter histological finding severity. This help us to select the efficacious therapeutic to treat the patients with LAM-resistant HBV.

PP-396

Non-invasive diagnostics of the hepatic fibrosis at early stages of its development (clinical supervision)

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The liver elastometry, Fibro-test and Doppler ultrasound of liver vessels were used for non-invasive clinic diagnostics of hepatic fibrosis. We present a clinical supervision of the patient with chronic viral hepatitis B for which 3 complementary methods of non-invasive diagnostics of fibrosis were executed simultaneously.

The patient P., 28 years was hospitalized in Vasilenko' Clinic in February, 2007. HBsAg was found at the patient for the first time of ambulatory inspection in 2000. No any visceral pathology was found at the survey. BMI was 28 kg/m2. The blood analyses showed the following: ALT was increased in 2 times, HBsAg+, HBeAg-, HBVDNA+, HBVDNA-7x104, HDVRNA-, markers of autoimmune and metabolic diseases of liver were negative. We made liver elastometry, Fibro-Acti test, gray-scale ultrasonography and Doppler ultrasound of liver vessels to determine the stage of fibrosis and activity of necro-inflammatory changes of liver tissue at the patient. The results of non-invasive tests showed the presence of weak liver fibrosis (F1) and low activity (A0-A1) at the patient that has been confirmed by data of liver biopsy: portal fibrosis without septs F1, A1 by METAVIR. The clinical diagnosis was formulated as follows: the chronic hepatitis B (HBVDNA +, HBeAg-), low activity (A1) and weak fibrosis (F1).

Thus, the applied methods of non-invasive diagnostics have shown high diagnostic accuracy and can be used independently, with the purpose of estimation of dynamics fibrosis at antiviral therapy.

PP-397

Serum HBV genotype testing and analyzing to the chronic and liver disease patients in Jilin Province

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The serum HBV genotype testing has been performed from 212 cases of chronic and liver disease patients with the positive HBVM in this province, the report is as the following: current research shows HBV genotype has a certain geographical area distribution, the testing result from 212 cases of chronic and liver disease patients with positive HBVM in the serum genotype is: 19 cases of B type (8.9%), 128 cases of C type (60.4%), 9 cases of D type (4.2%), 38 cases of BC type (17.9%), 15 cases of CD type (7.1%), and 3 cases of uncertainty (1.4%),

it means in Jilin Province the above types of HBV genotype are existing in the chronic and liver disease patients with positive HBVM, among which C type belongs to advantage genotype, closely with the reports from the south in China and the overseas. The difference distribution may be concerned with the infection path. It embodies the characteristics of variation in HBV infection history.

PP-398

Polymorphisms of the toll-like receptor 9 (TLR9) gene with hepatitis B virus infection in Chinese

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Objective: Chronic Hepatitis B virus (HBV) infection is the result of a complex interaction between a replicating noncytopathic virus and a down-regulated antiviral immune response.Toll like receptor (TLR)9 is a kind of pattern recognition receptors and producing large number of IFN-α by pDCs in response to virus is due to TLR9 recognition of CpG motif of virus. In our previous study, we have demonstrated that patients with chronic HBV infection have a various lower TLR9 expression of circulating pDCs. The aim of the present study was to elucidate whether the common variants in TLR9 gene were associated with the clinical types of patients with chronic HBV infection.

Methods: For selection of single nucleotide polymorphisms (SNPs), Haploview software (http://www.broad.mit.edu/mpg/haploview) was used to conduct linkage disequilibrium using Hapmap phase Ⅱ genotype data for the chromosomal region 3: 52,230,137-52,235,218 (CHB database, Hapmap release 21a [Jan.2007]). The amplicon of interest is a 5.082-kb region, with TLR9 and approximately 3 kb upstream and 3 kb downstream of TLR9. The selection of tagSNPs was performed by running the tagger program implemented in Haploview. The criteria for r^2 was set at >0.8. One tagSNP (rs187084) within TLR9 were studied in 209 patients including 130 patients with chronic HB and 79 HBV-related liver cirrhosis patients as well as 193 healthy controls.

Results: The frequencies of the C/C genotype and the C allele were increased in patients with HBV-related liver cirrhosis compared with healthy controls (26.6*vs.* 15.5%; OR,1.947, 95%CI, 1.045- 3.7 05, X^2=4.483, P=0.034/ 43.1 *vs.* 37.8%; OR, 1.485, 95%CI, 1.022-2.159, X^2= 4.32 3,P=0.038).

Conclusions: TLR9 gene polymorphisms were associated with the clinical types of patients with chronic HBV infection and may play an important role in the progress of HBV infection as one of the host factor.

PP-399

The analyse of effectiveness and safety in chronic viral hepatitis B treated by adefovir dipvoxil combined with bieyclol

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Objective: To analyze of the efficacy of adefovir dipivoxil combined with bicyclol in treatment of chronic hepatitis B (CHB) and determine its safty.

Methods: A total of 125 patients with CHB were randomized into the experimental group and the control group to be treated.The patients in the experimental group (63 samples) received adefovir dipivoxil orally 10mg daily and bicyclol orally 75mg daily for 48 weeks and those in control group (62 sample) received adefovir dipivoxil orally 10mg daily alone for 48 weeks. The serum aminotransferace (ALT/AST). HBV-DNA, HBeAg/antiHBe were observed before and after treatment. Results: compared with pre-treatment, The serum aminotransferace were all decreased obviously in two groups, the experimental groups is better (P<0.05, P<0.01).HBVDNA negative conversion rate was significantly higher in the experimental group than that in control group (58.7%vs40.3%,P<0.05). HBeAg loss rate was significantly higher in the experimental group than in control group (31.8%vs16.1%, P<0.05).Although the experimental group is higher than the control group in the aspect of HBeAg seroconversion rate,there were no statistical differrence between the two group.There were no obvious adverse events which were probably related to the drug in the study.

Conclusion: Adefovir dipivoxil combined with bicyclol is effective and safe in the treatment of chronic hepatitis B.

PP-400

The reconstituted human immune system and its ability of eliminating HBV by transplanting Human cord blood CD34+ cells in NOD/SCID mice

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Objective: To study the multilineage differentiation ability of Human cord blood CD34+ cells and its response to HBV.

Methods: Human cord blood(CB) CD34+ cells were transplanted into NOD/SCID mice post-irradiated by Co^{60} 3.5 Gy via tail vein,6×10^4per mouse. Human cells in mice peripheral blood（PB） were detected by flow cytometry at determined time points (2,4,6,and 9 weeks).4 weeks after transplanted, mice were infected HBV by human serum (contain HBV-DNA 6.8×10^7copy/ml，0.5ml per mouse**)** and detected HBV-DNA level at 1,7,10,and 15days.At the end of experiment (9 weeks), mice were sacrificed and the spleen were fixed by formalin and marked by LCA for observing human lymphocyte.

Result: Human cells differentiated from UCB CD34+ could be observed in NOD/SCID mice after transplanted. After 2 weeks, we could see CD3+CD8+T cells(18.6%),CD3+CD4+ T cells(16.1%),CD19+B cells(13.1%), CD56+NK cells, (27.8%).After infected by HBV-DNA, it could maintain 10^3 in 15 days in the mice without transplanted, and can be cleared in mice which transplanted with Human cord blood CD34+ cells.At 9wk，human CD45+ cells still could be detected in the spleen of transpanted mice by immunohistology.

Conclusion: CD34+ cells derived from human cord blood cells could rebuid human immune system in NOD/SCID mice, and could clear HBV.

PP-401

Cytokines fusion protein expression plasmid adjuvanted HBV DNA vaccination in mice

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The eukaryotic expression plasmid encoding the IL-2/IFN-γ fusion protein (pFP) was constructed to assess its adjuvant effect for in vivo electroporation (EP)-mediated HBV DNA vaccination in BALB/c and HBV transgenic (Tg) mice. The best response of either serum anti-HBs or HBsAg-specific IFN-γ T cells was achieved by pFP-assisted HBV DNA vaccination, the efficacy of which was affected significantly by withdrawal of pFP in BALB/c mice. The therapeutic efficacy of a HBV DNA vaccine was enhanced by pFP in that we observed the persistent suppression of HBV DNA replication and expression in Tg mice until the endpoint of the study when the more profound suppressive effects were accompanied by favorable INF-γ T cell responses and serum ALT elevation. The results suggest that IL-2 and IFN-γ, as the Th1-type cytokines in the form of a fusion protein expression plasmid, can enhance the potency of a HBV DNA vaccine.

PP-402

DNA double-strand break and its repair as a novel potential molecular mechanism for HBV integration

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HBV integration is present in up to 90% of HBV related HCCs and usually precedes the development of HCC even in young adults with non-cirrhotic liver, which strongly supports the oncogenic effect of HBV integration by itself. Nevertheless, the knowledge about the mechanisms of HBV integration is still in its infancy. Here we describe a potential mechanisms of HBV integration and delineate the possibility to rein in its process through regulating two kinds of gatekeeper proteins of DSB repair. Using I-Sce Ⅰ endonuclease-based system, we induced a DSB in the genome of human hepatoma cell line HepG2. When these cells were exposed to serum of chronic hepatitis B patients with high copies of HBV DNA, HBV DNA could be detected in integrated form at the exact site of DNA breaks. Furthermore, suppression of Rad52, the gatekeeper protein for HR repair pathway, by RNAi could remarkably increase the ratio of NHEJ to HR repair pathway, which, intriguingly, was accompanied by the elevation of the frequency of HBV integration. On the contrary, when Ku proteins, the gatekeeper protein for NHEJ repair pathway, were inhibited by RNAi,

the frequency of HBV integration was down-regulated accordingly. Thus, this project provided us with further evidence that DSB may serve as a potential and preferential target for HBV integration, and the process of HBV integration can be controlled by regulating the gatekeeper proteins of DSB repair, which highlight the feasibility to reduce the occurrence of HCC by suppression of HBV integration through adjusting two sorts of DSB repair pathways.

PP-403

Lamivudine-resistance multiple substitutions may decrease efficacy of add-on adefovir dipivoxil therapy in chronic hepatitis B patients

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Aims: To investigate characteristics of lamivudine-associated mutation in chronic hepatitis B patients who were not response to sequential therapy with add-on adefovir dipivoxil.

Methods: Sixteen lamivudine-resistance patients, without rt181 and rt236 mutations, who were not response to sequential therapy with add-on adefovir dipivoxil for six months were enrolled. HBV polymerase was analyzed by direct sequencing.

Results: More than three amino acid substitutions (range: 3-6) were demonstrated in these patients. Rt181 and rt236 substitutions were not found by direct sequencing. Other compensatory substitutions were showed, such as rt229 (4/16), rt173 (4/16), rt207 (3/16), rt109 (2/16), rt238 (2/16), rt80 (2/16), etc.

Conclusions: Lamivudine-resistance with multiple substitutions in HBV polymerase region may decrease the efficacy of sequential antiviral therapy with adefovir dipivoxil and lamivudine, even rt181 and rt236 mutations were not detected.

PP-404

Mechanism of defective interference by hepatitis B virus

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Human hepatitis B virus is a major human pathogen. Despite the fact that HBV vaccine is available, therapeutic treatment of HBV remains a big challenge. Chronic hepatitis B continues to be a major cause of end-stage liver disease and hepatocellular carcinoma worldwide. Naturally occurring HBV variants containing core internal deletion (CID) has been found geographically ubiquitous and highly prevalent in chronic HBV carriers. One important characteristics of this replication defective CID mutant is interference with the replication of homologous wild type helper virus and their ability to enrich their proportion in the total viral yield in cells infected with wild type and defective interference (DI) or incomplete CID mutant. Despite the extensive research on DI viruses, the molecular basis leading to a DI phenotype is unclear. Hereby we are focused on molecular mechanism of CID mutant interference. We found that the internally deleted core protein of CID variants does not function as a repressor of wild type virus replication. The preferential replication of mutant virus is dependent on the limiting wild type core protein as well as wild type polymerase. Over-expression of both core and polymerase from a plasmid can rescue the wild type virus. We also observed that host factors are also important for the preferential replication of CID mutant. This finding has important implications for chronic viral hepatitis and other chronic progressive viral diseases.

PP-405

Effect of lamivudine on IFN-γ and IL-4 in patients with chronic hepatitis B

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Objects: To investigated the effect of Lamivudine (LAM) on IFN-γ and IL-4 in patients with chronic hepatitis B (CHB).

Methods: Sixty-six HBeAg (+) patients with CHB were treated with LAM at a dose of 100mg/d. Serum levels of INF-γ and IL-4 were measured with enzyme linked immunosorbent assay (ELISA) at baseline and 3, 6, 9 and 12 months during the treatment. Serum indexes including liver function, HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc and HBV DNA were also measured. Twenty healthy volunteers were served as normal control.

Results: Compare with patients with low ALT levels, patients with high ALT levels showed higher IFN-r levels and higher IFN-γ/IL-4 ratios before LAM treatment（$p<0.05$，$p<0.05$）；Patients with high IFN-r levels showed much higher rate of complete response and lower rate of non response to LAM treatment than those with low IFN-r levels($P<0.01$).The IFN-γ/ IL-4 ratios in patients with complete response were approximate or even higher than those of control group. Whereas the IFN-γ/IL-4 ratios in patients with partial response and non response were lower than those of control group.

Conclusion: The CHB patients with better immune fuction show higher rate of response to LAM treatment. The treatment with LAM can increase the release of IFN-γ, whereas inhibit IL-4 levels. The response to LAM therapy is related to the balance of Th1/Th2. However, the treatment with LAM cannot induce general HBeAg seroconversion, combination of LAM and other immunomodulator may be a reasonable therapatic strategy of anti-HBV.

PP-406

The anti-fibrotic effect of traditional chinese medicine 319 recipe is associated with inhibition of intrahepatic expression of PDGF and TGF-β in rats.

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Introduction: Liver fibrosis is a consequence of severe liver damage that occurs in many patients with chronic liver disease. Traditional Chinese Medicine 319 Recipe (TCM 319) is a Chinese Medicine formula which consists of 6 Chinese herbs. In this study, we investigated the anti-fibrotic efficacy and mechanisms of TCM 319 Recipe.

Methods: Thirty-four male adult Sprague Dawley (SD) rats were allocated to 5 groups (group 1-concomitant CCl4 and TCM 319 for 8 weeks; group 2-CCl4 for 4 weeks and then CCl4 and TCM 319 for 4 weeks; group 3-CCl4 alone for 8 weeks; group 4-TCM 319 only for 8 weeks; group 5- untreated controls). After 8 weeks of treatment, serum and urine chemistry test, liver tissue histological examination, pico-sirius red staining and immunostaining were carried out to examine the liver function and fibrosis degree. The expression levels of platelet derived growth factor (PDGF-B), PDGF-Rβ, and transforming growth factor-beta 1 (TGF-β1) were measured by quantitative RT-PCR and western blot.

Results: When treated with CCl4 and TCM 319 after eight weeks, TCM 319 reduced the hepatic fibrosis area, collagen contents and α smooth muscle actin (α-SMA), compared with rats receiving CCl4 only. This was associated with the down-regulations of the mRNA and protein levels of PDGF-B, PDGF-Rβ, and TGF-β1($P < 0.05$).

Conclusions: TCM 319 recipe extracts could attenuate liver fibrosis in rats and inhibit the expression of PDGF and TGF-β.

Acknowledgment: This research is supported by UDM Departmental Research Grant, University of Hong Kong.

PP-407

Dysregulation of β-catenin by hepatitis B virus X protein in HBV-infected human hepatocellular carcinomas

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β-catenin is a key molecule involved in both cell-cell adhesion and Wnt signaling pathway. To evaluate whether dysregulation of β-catenin was correlated with hepatitis B virus X in hepatocellular carcinoma, β-catenin expression and transcriptional activity were determined in HBx stably tansfected HepG2 cells. As shown in Fig.1, the expression levels of wild-type β-catenin and E-cadherin were decreased in HepG2 cells expressing HBx. Furthermore, β-catenin dependent transcriptional activity was enforced in HepG2-HBx cells (Fig.2). Immunohistochemical (Fig.3) and Western blot analysis of β-catenin revealed that a decrease in the β-catenin protein level was found in 58.3% of HBV-related HCCs versus 19.2% of non-HBV-related tumors (Table.1). These data suggest that the expression of HBx contributed to the development of HCC, in part, by repressing the wild-type β-catenin expression and enforcing β-catenin-dependent signaling pathway.

Figures

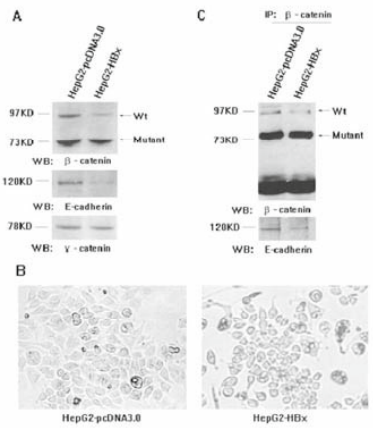

Fig.1

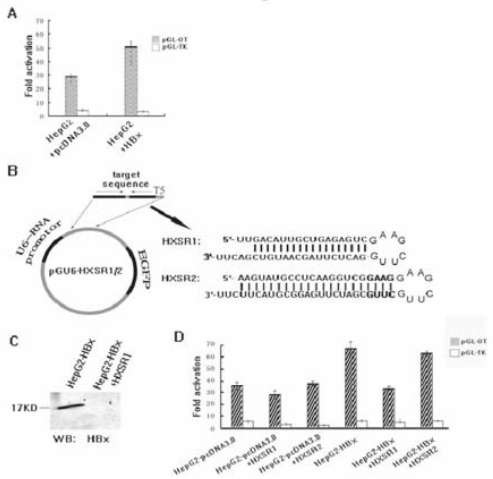

Fig.2

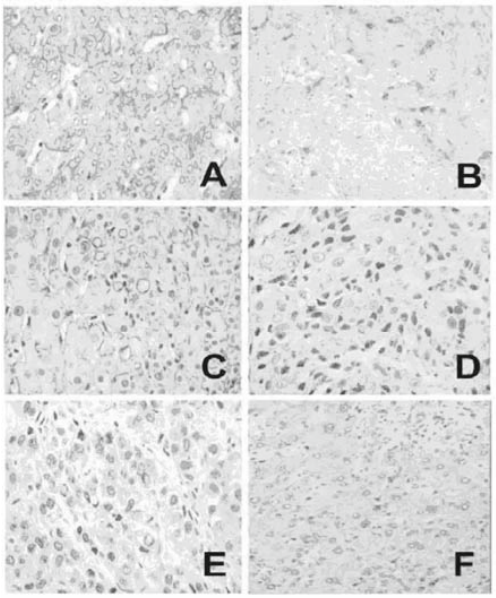

Fig.3

Table

Table.1 Correlations Between β-catenin Expression Levels and HBV Infection Status

	β-catenin			
	Decreased	Unchanged	Increased	
HBV(+)	35	14	11	
HBV(-)	5	14	7	P=0.0028
HBV(+)	35	25		
HBV(-)	5	21		P=0.0008

NOTE. β-catenin-decreased tumors include those with total loss of the protein.

PP-408

Function of the DC transfected with hepatic B core gene to stimulate self-lymphocyte in chronic hepatitis B patients

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Aims: To evaluate the effect of transfeced DC from chronic hepatitis B patients to excite self-lymphocytes.

Methods: PBMC form chronic hepatitis B patients were cultured and derivated into DC with GM-CSF and IL-4 . Hepatitis B core gene was transfected into DC by the liposme on the fifth day. Mix the self-lymphocyte into the DCs at the ration of 10 :1 ,then 5 days later, the secretion status of IFN-γ of the lymphocytes was measured by ELISPOT.Meanwhile,the mixed cells were used to kill the HepG2.2.15 cell in order to evaluate the CTL capability.

Results: Compared to the non-transfected DC, CD83 expression efficiency of the transfeced DC increased ($P<0.05$). The expression efficiency of HBcAg at 48, 72, 96 and 120 hours were 31.58%, 55.80%, 54.18% and 56.99% respectively. There was flavovirens fluorescence in pJW4303/HBc-DC group with fluorescence microscope,while no fluorescence in non-transfected DC group. The excited lymphocytes by transfected-DC could secret IFN-γ, While the controls can't. The HepG2.2.15 cells' death rates of experiment group were （62.5±4.8）%(E:T=10:1)、(71.8±5.3)% (E:T=20:1)、(81.5±5.0)% (E:T=40:1),while the controls only induced relatively lower cytotoxicity（$P<0.05$）.

Conclusions: The lymphocyte after excited by gene-transfected DC could secrete IFN-γ and could evoke a higher CTL response in vitro.

PP-409

Four-year treatment with entecavir results in high proportions of nucleoside-naive HBeAg-positive patients with undetectable HBV DNA: Results from studies ETV-022 and -901

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Background/Aims: The aim of chronic hepatitis B treatment is sustained suppression of HBV replication. 183 entecavir-treated patients from ETV-022 enrolled in the long-term rollover study ETV-901. We present efficacy and safety results in a cohort of patients from ETV studies -022 and -901 who received 4 years of continuous therapy with ETV.

Methods: The nucleoside-naive HBeAg (+) 4-year cohort consists of ETV-treated patients who completed ETV-022 and enrolled in ETV-901 with a treatment gap ≤35 days. In ETV-901, patients were treated with 1 mg of ETV. The proportions of patients with HBV DNA <300 copies/mL by PCR assay, ALT normalization, HBeAg loss or HBeAg seroconversion were evaluated among patients with evaluable samples at Week 192.

Results: 146 Patients from ETV-022 enrolled in ETV-901 with a treatment gap ≤35 days. Efficacy parameters for patients who completed 4 years of therapy were examined. Among patients with available samples, 98/108 (91%) achieved HBV DNA <300 copies/mL; 96/112 (86%) achieved ALT ≤1 × ULN; 39/96 (41%) experienced loss of HBeAg; and 15/96 (16%) experienced HBeAg seroconversion. By protocol design, most patients who achieved HBeAg loss during Years 1–2 discontinued study therapy. Numbers and proportions represent additional patients with HBeAg loss or HBeAg seroconversion at week 192.

Conclusions: At Week 192, 91% of patients who received continuous ETV treatment during 4 years had undetectable HBV DNA and 86% had ALT normalization. Patients continued to experience HBeAg loss and HBeAg seroconversion during Years 3 and 4. The safety profile was consistent with previously reported experience.

PP-410

Efficacy and safety of clevudine therapy in patients with hepatitis B virus-related liver cirrhosis

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Aims: Clevudine, L-FAMU, has a potent and sustained antiviral activity against HBV. It has been demonstrated that clevudine therapy showed potent antiviral effect in HBeAg-positive or negative hepatitis B patients. The aim of this study was to evaluate the antiviral and clinical efficacy of clevudine in CHB patients with cirrhosis.

Methods: Fifteen patients with HBV cirrhosis (HBV-DNA > 5 log10 copies/ml) treated with clevudine for at least 6 months were included. They received clevudine 30mg once daily. Biochemical tests and HBV-DNA level were checked at baseline and every 3 months during the treatment. Results: Eleven of the 15 patients were HBeAg-positive. Their mean age was 48.4 (31~65) years. All the patients had compensated cirrhosis, and four patients had HCC. Baseline median HBV-DNA level was 6.3 log10 copies/ml, and their mean ALT level and total bilirubin were 184 IU/L and 1.3 mg/dl. Median duration of the clevudine therapy was 36 weeks (24~52 weeks). Ten of the 15 patients (67%) achieved undetectable level of HBV-DNA (< 300 copies/ml) and 9 patients (60%) had normal ALT after 24 weeks of the treatment. Median serum HBV DNA reductions from baseline at week 12 and 24 were 2.9 and 3.7 log10 copies/ml, respectively. No one developed hepatic decompensation or HCC during the treatment. There was no adverse event.

Conclusions: Clevudine is safe and has a rapid and potent antiviral effect in cirrhosis patients. The antiviral efficacy in the patients with cirrhosis is comparable to those in chronic hepatitis B patients.

PP-411

Inhibition of hepatitis B virus replication by APOBEC3G in vitro and in vivo

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To investigate the effect of APOBEC3G mediated antiviral activity against hepatitis B virus (HBV) in cell cultures and replication competent HBV vector-based mouse model.

The mammalian hepatoma cells Huh7 and HepG2 were co-transfected with CMV-driven expression vector encoding APOBEC3G and replication competent 1.3 fold over-length HBV. Levels of HBsAg and HBeAg in the media of the transfected cells were detected by ELISA. The expression of HBcAg in transfected cells was detected by western blot. HBV DNA and RNA from intracellular core particles were examined by Northern and Southern blot analyses. To assess activity of the APOBEC3G in vivo, an HBV vector-based model was used in which APOBEC3G and the HBV vector were co-delivered via high-volume tail vein injection. Levels of HBsAg and HBV DNA in the sera of mice as well as HBV core-associated RNA in the liver of mice were determined by ELISA and quantitative PCR analysis respectively.

There was a dose dependent decrease in the levels of intracellular core-associated HBV DNA and extracellular production of HBsAg and HBeAg. The levels of intracellular core-associated viral RNA also decreased, but the expression of HBcAg in transfected cells showed almost no change. Consistent with in vitro results, levels of HBsAg in the sera of mice were dramatically decreased. More than 1.5 log10 decrease in levels of serum HBV DNA and liver HBV RNA were observed in the APOBEC3G-treated groups compared with the control groups.

These findings indicate that APOBEC3G could suppress HBV replication and antigen expression both in vivo and in vitro , promising an advance in treatment of HBV infection.

PP-412

Dynamic changes of HBV quasispecies in the early stage of lamivudine treatment might predict the antiviral efficacy

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Objective: To investigate dynamic changes of HBV quasispecies within RT region in early stage of lamivudine treatment and its prediction for antiviral efficacy.

Methods: 8 chronic hepatitis B patients received lamivudine treatment for 48 weeks. Five patients responded to lamivudine, and three patients were nonresponders who developed resistance to lamivudine. HBV DNA from serum samples of baseline and week 4 were extracted. RT region of HBV genome was amplified, and then TOPO cloned and sequenced. Quasispecies complexity and diversity within RT region in baseline and week 4 were analyzed, and viral nucleotide substitution rates during first four weeks were calculated.

Results: Quasispecies complexity and diversity of responders in week 4 were （0.24±0.22）and（1.18±1.16）$\times 10^{-3}$ substitution/site, respectively, which were significantly lower than those of nonresponders （0.90±0.11） and （4.29±1.31） $\times 10^{-3}$ substitution/site respectively, ($P<0.05$), whereas quasispecies complexity and diversity showed no difference between responders and nonresponders at baseline. The dynamic change of quasispecies complexity and diversity was correlated with HBV DNA change in early stage (r=0.867 and 0.890, respectively, $p<0.01$). Viral nucleotide substitution rates of responders [(7.14±3.19) $\times 10^{-4}$ substitution/site/week] was significantly higher than that of nonresponders [(1.50±0.8) $\times 10^{-5}$ substitution/site/week] ($P<0.05$).

Conclusion: The dynamic changes of HBV quasispecies within RT region showed different patterns between responders and non-responders in early stage during lamivudine treatment and might predict the lamivudine efficacy, which mechanism needs to be further investigated.

PP-413

Adaptive evolution of hepatitis B virus preC/C driven by host immune selection stress

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Proteins (HBc and HBe) encoded by HBV preC/C (ORF C) play essential roles in viral capsid assembly and genome maturation. These important functions of preC/C determine that the majority of codons of ORF C are constrained by purifying selection for the maintenance of viral infectivity. On the other hand, these proteins are highly immunogenic and are recognized by host surveillance. Therefore, the amino acid changes resulting in viral escape from the host immune system would also be at a selective advantage. Such evolutionary changes will be promoted by positive selection. The selection that drives sequence variation in preC/C gene of HBV has been poorly understood. In this study we carried out condon-based maximum likelihood (ML) analysis to detect positively selected sites at the level of individual condons and to explore the possible evolutionary advantage of the amino acid variability. Our results revealed a strong correlation between selected sites and the known epitopes on core protein. Of the twenty five distinct sites, twenty one were mapped on epitopes of B-cell, cytotoxic T-lymphocyte and T-helper cell, respectively. This finding provides evidence for the adaptive evolution of preC/C of hepatitis B virus driven by host immune selection stress.

PP-414

Patterns of serum HBsAg kinetics in chronic hepatitis B patients with rapid and good viral responses to long term lamivudine treatment: a retrospective study

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Backgrounds/aims: To observe serum HBsAg kinetics in CHB patients with lamivudine therapy.

Methods: Forty five consecutive patients were studied, which were HBeAg positive and naive to antiviral therapy prior to lamivudine treatment then achieved rapid and good viral responses at week24 and till to week156. Abbott Architect HBsAg assay was used to quantify serum HBsAg. HBV genotypes were determined by direct sequencing.

Results: Twenty five (57.8%) patients gained HBeAg loss during the observation (figure 1A) and HBsAg lost in 1 patient. Serum HBsAg decreased to 39.5% (median) at week12 ($p<0.001$) taking baseline levels as 1.0, and no more reductions were found afterwards (figure 1A). More tremendous HBsAg reductions in first 12 weeks occurred in genotype B patients (n=21) than in C ($p<0.001$, figure 1B) but HBsAg levels at week156 were not different ($p=0.111$). HBsAg changes exhibited two distinct patterns: biphasic pattern (n=25, figure 1C) and assurgent pattern (n=14, figure 1D), which was associated with HBV genotypes (B/C) ($p=0.024$), however not with HBeAg loss ($p=1.000$).

Conclusions: HBsAg changes, presented as biphasic decline or assurgent pattern, might explain the rare occurrences of HBsAg loss with long term lamivudine therapy.

PP-415

Dynamic changes of HBV qusispecies and deletion patterns in a chronically infected patient

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Background & Aims: Sequential antivirus therapy may lead to the selection of drug resistant mutants. In addition to point mutations, deletion may also be selected during drug treatment. To investigate this process, we analyzed time point samples from a patient who failed lamivudine therapy and was subsequently treated with adefovir dipivoxil.

Methods: HBV DNAs extracted from eight samples were clone sequenced at a region of 1.5 kb including C and partial preS gene.

Results: In all samples, wild-type were co-existed with deletion mutants. Significant differences were found in the quasispecies distribution and deletion patterns between two antivirus therapies. During lamivudine treatment, wild-type strains (57.7%) were dominant, deletions of 15bp, 54bp and 129bp in preS were commonly observed. However, in following adefovir dipivoxil treatment, a virus population harboring 81bp and 96bp deletions (86%) became prevailed, and the wild-type only left as 14%. Interestingly, both two major deletions located in C gene encoded epitopes. Meanwhile, the frequency of preS deletions was significant decreased or disappeared except for the 129bp deletion. Notably, deletions in C gene were always accompanied with certain substitutions. The 81bp and 96bp deletions were fully correlated with 550G(A) and 422T(C) respectively.

Conclusions: Our study revealed a complex variation in quasispecies composition and deletion patterns during sequential therapies. The mutants with deletion at epitopes encoded by C gene overtook the wild-type and became dominant strains, suggesting possible immune escape. The correlation between C gene deletion and substitution may provide new insight on the research of deletion mechanism.

PP-416

Surface protein mutations in chronic hepatitis B patients who received two different forms of HB vaccine therapy

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Aims: The aim of this study was to determine the correlation between vaccine therapy and mutation in HBsAg positive chronic patients.

Methods: 11 and 4 patients received Engerix-B and Heberbiovac, respectively. The surface gene was amplified and directly sequenced from samples prior to vaccination and six months after the third dose.

Results: Only one patient responded. Of the total of 92 amino acid changes, 51 occurred in immune epitopes: 5 were in B cell, 21 in T h, and 25 in CTL epitopes.

Conclusion Mutations in immune epitopes could be escape mutations which are responsible for nonresponsiveness to vaccine therapy.

PP-417

Hepatitis B vaccination of chronic HBV infected individuals who lost HBsAg during follow-up

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From April 1993 to December of 2005, among 1603 chronic HBV infected individuals, 34 (2.1%) subjects (22 males,12 females) who became HBsAg and anti-HBs negative received HBV vaccine followed with the second and third doses at 1, and 6 months (group 1).All of these cases were HBV DNA negative. Fifty-two healthy cases (30 males, 22 females) who were negative for HBsAg, anti-HBs and anti-HBc also received HBV vaccine as above schedule (group 2). Post vaccination tests for HBsAg, anti-HBs were assessed two months of the later dose in both groups. The mean age of group 1 was 38±12.7 and in group 2 was 33.4±8.6 years ($p=0.07$). The distribution of sexes between these two groups were similar ($p=0.652$).The mean duration of follow-up for group 1 was 7.6±4.5 years (range 2 to 20 years).The mean anti-HBs antibodies level in group 1 (8 cases) was 277±324 (range 10 to 956) and in group 2 (45 cases) was 397±265 (range 35 to 950) mIU/ml ($p=0.258$).Anti-HBs levels ≥10mIU/ml were seen in 8 (23.5%) subjects in group 1 and in 45 (86.5) cases in group 2 ($p=0.00001$).The results show that nearly 24% of chronic HBsAg positive cases who lost HBsAg responded to hepatitis B vaccine and remaining of these cases need to be followed as cases of occult HBV infection.

PP-418

96 weeks of entecavir (ETV) re-treatment of HBeAg(-) ETV patients who previously discontinued treatment results in high proportions of patients with undetectable HBV DNA

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Background/Aims: The majority of HBeAg(-) nucleoside naive patients enrolled in ETV study-027 met protocol-defined criteria of response at Week-48 and discontinued treatment. Discontinuation of successful ETV treatment resulted in rising HBV DNA and ALT levels.

We report 96-week results of ETV re-treatment in a cohort of ETV-treated patients from study ETV-027.
Methods: The HBeAg(-) nucleoside naive ETV re-treatment cohort consists of patients initially treated with ETV in ETV-027 who subsequently enrolled in ETV-901 with a treatment gap of >60 days between the last dose in ETV-027 and the first dose in ETV-901. In ETV-901, patients were re-treated with 1 mg of ETV. The proportions of patients with HBV DNA <300 copies/mL by PCR assay and ALT normalization were evaluated among patients with evaluable samples at Week-96 of ETV re-treatment.
Results: Ninety-nine patients enrolled in ETV-901 with a treatment gap >60 days between ETV-027 and ETV-901. Efficacy parameters after 2 years of ETV re-treatment were examined. At 96 weeks, among patients with available samples, 67/74 (91%) patients had achieved the endpoint of HBV DNA <300 copies/mL and 60/76 (79%) of patients achieved ALT ≤1 × ULN. ETV's safety profile was consistent with the previously reported experience.
Conclusions: At 96 weeks of ETV re-treatment, 91% of HBeAg(-) patients who previously discontinued ETV therapy had undetectable HBV DNA and 79% had ALT normalization.

PP-419
Entecavir monotherapy is effective in the treatment of lamivudine- and adefovir-refractory chronic hepatitis B patients, except those with pre-existing YVDD (rtM204V) mutation.
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Entecavir is one of the drugs of choice in lamivudine-refractory hepatitis B patients due to previously demonstrated potent antiviral activity against lamivudine-resistant HBV. We prospectively studied the efficacy and durability of HBV suppression in patients who were switched to entecavir after failing lamivudine therapy from a single Asian tertiary centre. 14 Chinese patients who were refractory to lamivudine therapy were recruited, 12 of whom were refractory to both lamivudine and adefovir (defined as HBV DNA>10^5 copies/ml after at least 24 weeks of treatment). Mean baseline HBV DNA was $6.0x10^7$ copies/ml. All were either non-cirrhotic or had well-compensated cirrhosis with Child-Purgh scores of <7. 12 of the 14 patients were HBeAg positive. Patients were reviewed regularly for a mean duration of 26.3 months. One passed away at 15th month from severe cholangitis and another stopped treatment after being diagnosed with intracranial lymphoma at 17th month. All but 4 patients had good virologic response to entecavir, with mean HBV DNA $1.0x10^3$ copies/ml (reduction of >4 logs). All 4 patients with virologic rebound on entecavir (mean HBV DNA $1.5x10^7$ copies/ml) had previously documented YVDD (rtM204V) mutation. The entecavir responders had YIDD (n=7), adefovir resistance mutation (rtA181V; n=2), and YVDD (n=1; deceased at 15th month). Only 1 patient had persistent ALT elevation, who was a poor responder to entecavir. These findings suggest that hepatitis B patients who were refractory to lamivudine and adefovir can be treated with entecavir monotherapy, except for those with YVDD mutation, who may benefit from combination therapy from the start.

PP-420
Genetic mutation analysis of HBV precore/basal core promoter in patients with acute-on-chronic hepatitis B liver failure
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Objective: To characterize the property of HBV precore/basal core promoter (BCP) mutations in patients with acute-on-chronic hepatitis B liver failure (ACLF).
Methods: Sera from 341 patients with ACLF and 415 patients with chronic hepatitis B (CHB) were collected. HBV precore/BCP gene fragment was amplified by nested PCR and analyzed by direct DNA sequencing. Substitutions at 10 sites and insertion/deletion in the region were analyzed. HBV genotyping were performed by S-gene sequencing.
Results: The mutation-negative frequency was significantly lower in ACLF patient than in CHB patients (3.8% vs. 27.0%, $P < 0.01$). Substitution occurrences at the 7 of 10 sites were significantly higher in ACLF patients than in CHB patients (25.2% vs. 12.5% at T1753, 80.1% vs. 51.6% at A1762, 85.0% vs. 53.7% at G1764, 11.1% vs. 4.8% at C1766, 6.5% vs. 2.7% at T1768, 51.0% vs. 32.0% at G1896, and 22.9% vs. 6.7% at G1899). The substitution occurrences at the other 3 sites (T1754, T1758 and G1862) were all less than 5% for both ACLF and CHB patients. ACLF patients had a remarkable increase of multiple substitutions at the 10 sites than CHB patients, with 58.4% vs. 34.5% ($P < 0.01$) for ≥three-site substitutions, 30.8% vs. 12.5% ($P < 0.01$) for ≥four-site substitutions, and 10.9% vs. 2.4% ($P < 0.01$) for ≥five-site substitutions. In addition, insertion/deletion mutations were more frequently observed in ACLF patients than in CHB patients.
Conclusion: Mutation accumulation in HBV precore/BCP region is associated with acute-on-chronic liver failure on the basis of chronic hepatitis B.

PP-421
Prognosis after HBsAg disappearance and response to hepatitis B(HB) vaccine and combined HB vaccine with immunological stimulator in populations infected HB virus.
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Aim: To investigate the prognosis after HBsAg disappearance and the response to rHB vaccine and combined rHB vaccine with immunological stimulator in populations infected HBV.
Methods: 114 subjects were consecutively collected and divided into three groups since 2001 between which were comparable in sex and age. Group A involved 56 cases,21 of them from group B who had not response to rHB vaccine alone, were vaccinated intradeltoideusly with 30μg rHB vaccine weekly and injected subcutaneously with 1.6 mg thymosinα1 (Tα1) twice a week for four weeks. Group B contained 33 cases received rHB vaccine alone as group A. Group C contained 25 cases were regularly tested but without any medicines.
Results: The anti-HBs seroconversion rates in subjects with all HBV marks negative, anti-HBe and anti-HBc positive, or anti-HBc positive were 93.3% (14/15), 73.7% (14/19), 68.2%(15/22)in group A, and 11.1%(1/9), 0%(0/11), 0%(0/13)in group B($p<0.001$), respectively, and had not change in group C(0/6,0/8 and 0/11).The seroconversion rate(61.9%)of anti-HBs was also higher in 21 subjects from group B than other subjects in group B and C($p<0.001$). Only 3 cases (7.3%) in group A anti-HBc disappeared during a mean 3.4 years following up. Anti-HBs seroconversion were each 1 cases in group A, B C during following up 2-5 years. HBsAg reversion were 1 case each in group B and C 18 and 26 months after the immunization.
Conclusion: There is a low natural anti-HBs seroconversion rate over 3 year after HBsAg disappearance, even may reverse to HBsAg. It is effective for those who have not anti-HBs after HBsAg disappearance to obtain high anti-HBs seroconversion rate, especially in those whose HBV marks are fully negative, within a short time, by using rHB vaccine plus Tα1 immunological intervene.

PP-422
Development and utility of a rapid method for detection of PreS2 mutants of hepatitis B virus
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PreS2 mutants of hepatitis B virus (HBV) contain small in-frame deletions within the up-stream region of the preS2 domain of the surface antigen gene; some of these mutants also have a missense mutation of the middle surface antigen start codon. Investigators have shown that HBV-infected people with hepatocellular carcinoma (HCC) are much more likely to be infected by preS2 mutants than those without HCC. Furthermore, recent data suggest that the presence of these mutants in HBV-infected people may be a marker for increased risk of HCC. Thus, a sensitive, rapid, and reproducible method of detecting these mutants will be very useful for further research on this topic. Towards this goal, we have modified a PCR-based method developed by Raimondo et al (1). Primers spanning the preS2 domain, one of which is fluorescently labeled, are used to amplify HBV DNA purified from patient sera. A portion of each amplified mixture is then cleaved with NlaIII. Aliquots of the PCR products, both before and after digestion, are directly loaded onto an Applied Biosystems 3130xl Genetic Analyzer, for determination of the size of the fluorescently labeled products. Both deletion and missense mutations, even when present in a mixed population, can be easily and sensitively detected using this method, which also allows high throughput. Analysis of patient sera at various stages of chronic hepatitis B is on-going, and the data will be presented at the Congress.
Reference:
1. Raimondo G, Costantino L, Caccamo G, et al. J Hepatol. 2004; 40:515-9.

PP-423

Inhibitory fffect of intracellular anti-HBc ScFv on HBV

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Aim: To study the effect of intracellular anti-HBc ScFv on HBV replication.

Method: Anti-HBc ScFv gene was cloned into adenoviral shuttle vector pAdTrack-CMV and recombined with adenoviral backbone vector pAdEasy-1.then the recombinant adenoviruses Ad-ScFv were produced in 293 Cells. HepG2.2.15 cells approximately 10^8 in $25cm^2$ flasks were infected with 10 μl viral stocks at a MOI≈10. the medium was removed 24 hours after infection. Subsequently, the medium was changed on day 3, 5, 7, and 10 after infection, and HBV DNA, HBeAg, and HBsAg in supernatant of the medium were detected by real-time fluorescent quantitative PCR and IMX assay respectively.

Result: 24 hours after infection with adenoviruses, GFP expression was observed in almost all HepG2.2.15 cells, and the growth of HepG2.2.15 cells was not affected after infection. HBV DNA in the culture medium of HepG2.2.15 cells had not decreased apparently as compared with the control cells without infection, but the levels of HBeAg and HBsAg in the culture medium were decreased gradually after infected with adenoviruses as compared with the controls.

Conclusion: Intracellular anti-HBc ScFv has not toxic effect on HepG2.2.15 cells and obvious effect on HBV DNA replication, but can inhibit the expression of HBV.

PP-424

The accumulation of deleted HBV in preS region during antiviral medication and the distribution pattern of HBV deletion in northern China

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From HBV sequences of 51 whole genomes and 70 preS clones from 103 patients in Beijing, we observed three hot spots of deletions including core, preS, and BCP regions. Most core and BCP deletions were out of frame, whereas almost all preS truncations were in frame. Most deletions interrupted epitopes of viral proteins, suggesting possible mechanism for virus to evade immune surveillance. Furthermore, among various clinical issues, by logistic regression only antiviral medication appeared to affect deletion accumulation (OR =6.81, 95% CI 1.296～35.817, P=0.023). In 33 full length sequences from asymptomatic carriers and chronic hepatitis individuals, significantly higher rate of deletion was seen in antiviral treatment group in comparison with the untreated (FET, P=0.007). Particularly, much higher frequency of preS2 deletions was found in nucleotide analog treated group (FET, P=0.023). Supporting evidence also came from time point samples. PCR and clone sequencing demonstrated the increase of deletions at preS1 or preS2 in quasispecies of 2 patients with ADV medication for three months, but not in samples of untreated individuals. Meanwhile, preS deletions exhibited complex patterns and were classified into 4 major types, among which type IV (internal deletion of preS2) was the most common (37%).Conclusion: We present here the panorama of deletion distribution patterns and preS deletion substructures in genomes of HBV prevailing in northern China. NA medication may contribute to the accumulation of preS deletion mutants. Investigation on the correlation of deleted sequences in preS region to viral survival and functions is in the process.

PP-425

Specific geographical and ethnical distribution of HBV CD recombinants in Western China

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Two forms of recombinants between HBV genotype C and D was identified in western China. But their geographical and ethnical distributions, as well as the mechanism of recombination have not yet been fully studied. In this study, 1024 chronic HBV carriers were enrolled from four ethnical nationalities (Han, Tibetan, Hui and Uygur) representing five provinces (Tibet, Qinghai, Gansu, Ningxia and Xinjiang) of Western China. HBV genotypes were determined with PCR-RFLP. Patients infected with genotype D and C/D recombinant, and selected samples with genotype B and C were confirmed by nucleotide sequencing. The distribution of HBV genotypes was as follows: HBV/B: 83 (8.1%); HBV/C: 438 (42.8%); HBV/D: 45 (4.4%); HBV/CD: 458 (44.7%). They were identified at distinct pattern in different regions and ethnical populations. HBV/CD1 recombinant was found in all five provinces, with a much higher prevalence than HBV/CD2 recombinant in Qinghai, Gansu and Ningxia provinces. While in Tibet, CD2 strain was a little higher than CD1. Geographically, the distribution of CD recombinants in western China showed an increasing gradient from northeast to southwest, while genotype C showed a decreasing tendency among the patients. Ethnically, CD recombinants had a remarkable high prevalence in native aboriginal Tibetan patients than in local Han patients. Our results demonstrated that HBV CD recombinants restricted in a specific region in western China, mainly in Qinghai-Tibet Plateau. Therefore it is suggested that the recombinants are the consequence of adaptation of HBV to certain genetic and immunologic characteristics of population instead of recombination between genotypes C and D.

PP-426

A study of the quantitative method for detection hepatitis B virus covalently closed circular DNA in serum

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Objective: To establish a quantitative method for detection hepatitis B virus covalently closed circular DNA(HBV cccDNA) in serum.

Methods: According to the structural difference between HBV cccDNA and rcDNA, two primers and a Taqman probe were designed. The HBVDNA was extracted in serum from patient with chronic hepatitis B and digested by Plasmid-Safe ATP-Dependent Dnase(PSAD) for removing HBV rcDNA. A reconstructed plasmid was used to standard. A quantitative method for detection HBV cccDNA in serum was established by real-time fluorescent PCR. For evaluation the specificity of method, the serum extractions before or after digested by PSAD were amplified and the variation of cycle threshold (Ct) was observed. A series of dilution plasmids were amplified several times for deciding sensitivity and repeatability of method. 57 sera collected from patients were detected HBV cccDNA.

Results: A quantitative method for detection HBV cccDNA in serum was established successfully byeal-time fluorescent PCR. The detectable linear range was from 1×10^3copies/ml to 5×10^8copies/ml and its sensitivity was 10^3 copies/ml. The variation of Ct before and after digested by PSAD was no difference in sera or plasmids .The coefficient variation (CV) was lower than 5% when plasmids were repeatedly detected. Of 57 sera, 30 (52.6%) were detected HBV cccDNA. There was no statistically significant among the detective rate of HBV cccDNA, HBVDNA load and HBeAg tsatus.

Conclusions: The real-time fluorescent quantitative PCR method for detection HBV cccDNA in serum has higher sensitivity, specificity and repeatability. It can be used in clinical research.

PP-427

Enhancing the immune responses of HBsAg-pulsed DC vaccine to hepatitis B virus by blocking PD-1:PD-L1 signal

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Mechanisms contributing to chronic hepatitis B virus infection are not well understood. Impaired function about DCs in chronic viral infections has been reported. Recent reports showed that PD-1 and its ligand PD-L1 interaction had negatively adaptive to T lymphocytes function. To observe the immune responses of DC vaccine to hepatitis B virus after blocking PD-1:PD-L1 pathway by PD-L1 monoclone antibodies(Abs). We treated HBV transgenic mice (inbred BALB/c) with blocking Abs specific for PD-L1, and followed by adoptive transfer HBsAg-pulsed DCs, which were generated from bone marrow cells from BALB/c mice. Following three and six days after transfer, we showed that blocking PD-L1 signaling stimulated splenic $CD3^+CD8^+$ T lymphocytes proliferation more efficiently than no PD-L1 blockade (mice treatment with DC/HBsAg alone), and high capability of secreting IFN-γ, which was an important antiviral cytokine. In addition we found that the HBsAg titers in serum of mice treatment with PD-L1 Abs were distinctively decreased and a concomitant ALT levels increased. These results indicate that blocking PD-1:PD-L1 interaction can enhance specific $CD3^+CD8^+$ T lymphocytes proliferation and capability of secreting IFN-γ, which improves function of inhibiting HBV in transgenic mice induced by HBsAg-pulsed DC vaccine.

PP-428

Study on the efficacy of interruption of maternal-infantile transmission of HBV of 1269 cases

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Objective: To investigate the best protective method of maternal-infantile transmission of HBV.

Method: From 1985 to 2004,1269 infants from HBsAg（+）mother received various protective methods and were asked to have blood samples collected at 0,1,6,12 month after birth respectively. They were divided into 5 groups according to 5 different protective methods as follows: plasma-derived HBV vaccine (the first)group and combined HBIG(the second) group, yeast recombinant HBV vaccine (the third) group and combined HBIG(the forth) group, intrauterine interruption(the fifth) group. Infants were followed up to 12 months of age.

Result: The positive rate of HBsAg (3.86%) of the fifth group Was lower while the positive rate of Anti-HBs(96.14%) was higher than that of the other 4 groups. Compared with the first and the third group , the second and the forth group , the two positive rates also showed significant difference P<0.01. The incidance of intrauterine HBV infection in the infants of the fifth group were much lower than that of the forth group (32.0%、 51.7%). The protective efficacy of the second and fifth group was better than that of the first and third group, P<0.05.

Conclusion: Among the five methods, the fifth group was most effective, vaccine combining HBIG was prior to vaccine only. The maternal-infantile transmission of HBV was relation to mothers' positiveness of HBV-DNA.

PP-429

The study on the relationship between the C gene mutations of HBV and HBVDNA rebound after Lamivudine therapy

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Objective: To investigate the relationship between the C gene mutations of hepatitis B virus (HBV) and HBVDNA rebound after lamivudine therapy.

Methods: 10 patients of CHB with HBV DNA rebound after lamivudine therapy(therapy group) and 10 patients of CHB without any antiviral therapy (control group) were investigated. The Core gene mutation sites were identified by PCR gene sequence analysis and HBV genotype was detected by nested PCR with multiple pair primers in sera of 10 patients after HBV DNA rebound and in sera of 10 patients in the control group. .

Result: Core gene mutation sites included I134V ，P164Q ，I126L+E142D+P159T，L84I+I88V+I126L+P159A+P164 were found in the 4 patients of therapy group ,and C Core gene mutation sites included A83G，A83G+P164Q ，I126L+P164Q ，I126L+P159T were found in 5 patients of in control group，The mutation sites involved in I134V、E142D，L84I and I88V were only detected in therapy control group（3 cases） all of whom were infected with HBV genotype C.

Conculusion: The mutation sites of I134V、E142D，L84I and I88V in C gene may be associated with lamivudine resistance; The C gene mutations refered to lamivudine resistance occurs more frequently in the patient with HBV genotype C than in those wih HBV genotype B.

PP-430

Evaluation of peripheral blood T-lymphocyte subpopulations in various clinical phases of chronic hepatitis B virus infection: relationship with viral load

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Aim: To investigate peripheral T-lymphocyte subpopulation profile and its correlation with viral replication in patients with chronic HBV infection.

Methods: Distribution of T-lymphocyte subpopulations in peripheral blood was measured by flow cytometry in 422 chronic HBV-infected patients and 100 controls. HBV markers were detected with ELISA and viral load with quantitative real-time-PCR. The relationship between HBV replication and variation in peripheral T cell subsets was analyzed.

Results: Chronic HBV-infected patients had significantly decreased $CD3^+$, $CD4^+$ cells and $CD4^+/CD8^+$ ratio, and increased $CD8^+$ cells compared with non-infection controls. There was a significant linear relationship between viral load and these parameters of T-lymphocyte subpopulations (linear trend test P<0.001). In immune-tolerant- and immune-active phases the peripheral blood contained more $CD8^+$T cells than $CD4^+$T cells, whereas this was the opposite in inactive carriers and normal controls. This T cells impairment was also significantly associated with high viral load respectively. In multiple regression, both in the patients of immune-tolerant phase and of immune-active status, after adjustment for all other variables, log copies of HBVDNA maintained its highly significant predictive coefficient on T-lymphocyte subpopulations, and was the strongest predictor in variation of $CD3^+$, $CD4^+$, $CD8^+$ cells and $CD4^+/CD8^+$ ratio, whereas the effect of HBeAg was not significant.

Conclusion: T-lymphocyte impairment was significantly associated with viral replication level. The substantial linear dose-response relationship and strong independent predictive effect of viral load on T-lymphocyte subpopulations suggests the possibility of a causal relationship between them, and indicates the importance of viral load in the pathogenesis of T cell hyporesponsiveness.

PP-431

Practical value of serum albumin and globulin for diagnosing hepatitis B associated cirrhosis

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To appraise the practical value of serum albumin (ALB) and globulin (GLB) levels and albumin/ globulin (A/G) ratios for diagnosing hepatitis B associated cirrhosis.

Methods: In 172 patients with chronic hepatitis B, 28 patients and 144 patients were diagnosed as cirrhosis and non-cirrhosis respectively by pathological method. SPSS 12.0 was used for statistical analyses. Serum ALB and GLB levels and A/G ratios for diagnosing cirrhosis were appraised with ROC curve. **Results**: The areas under ROC curve of serum ALB levels and A/G ratios for diagnosing non-cirrhosis were 0.221（95%CI：0.138～0.304） and 0.229 (95%CI：0.133～0.326）, the area under ROC curve of serum GLB levels for diagnosing cirrhosis was 0.720（95%CI：0.617～0.824）. The optimal cut-offs of serum ALB and GLB levels and A/G ratios for diagnosing cirrhosis were 40.35 g/L and 34.70 g/L and 0.98, respectively; the sensitivities, specificities, positive predictive values, negative predictive values, accuracies, Youden's indexes of the optimal cut-offs were 0.821 and 0.607 and 0.571，0.646 and 0.771 and 0.868，0.311 and 0.340 and 0.457，0.949 and 0.910 and 0.912，0.674 and 0.744 and 0.818，0.467 and 0.378 and 0.439.

Conclusions: Serum ALB, GLB levels and A/G ratios can all be used for diagnosing negatively cirrhosis.

PP-432

The clinical and histological features of HBV carriers with persistently normal serum ALT levels (PNAL)

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In view of the findings that 20~40% HBV carriers with PNAL have significant histological finding (SHF), the clinical and histological features of eAg(-) and eAg(+) carriers with PNAL are uncertain. We divided subjects with PNAL who had had liver biopsy into 2 groups according to their e antigen status. Each group was further split into two subgroups: SHF and non-SHF group. Demographic, biochemical, virological variables were evaluated.81 eAg(-) and 87 eAg(+) carriers met our inclusion criteria, each had 23.5% and 24.1% subjects with SHF. Subgroup analysis showed that SHF was associated with increasing age in both groups (P=0.05 for eAg(-), P=0.00124 for eAg(+)) and the cut off value was 45yrs for the former and 30yrs for the latter. In eAg(-) group, SHF was also associated with increasing HBV-DNA levels (P=0.032) with the cut off value of 10^4 copies/ml and higher normal ALT level with the cut off value of 30U/L (P=0.046 for the whole and 0.014 for male). In eAg(+) group, SHF was associated with decreasing HBV-DNA level (P=0.001) with the cut off value of 10^8 copies/ml(P=0.010).Conclusion: For eAg(-) carriers with PNAL,

liver biopsy is necessary if their age ≥45yrs, HBV-DNA ≥10^4 copies/ml or they are male patients with ALT>30U/L. For eAg(+) ones, high HBV-DNA level may indicate the immune-tolerant phase.

PP-433

Pretreatment alanine transaminase level as a predictor of HBeAg loss in older patient

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Elevated pretreatment alanine transaminase (ALT) level over 2 times the upper limit of normal (ULN) reference range has been accepted as the marker, predicting HBeAg loss and thus the relevant indicator for initiating the antiviral therapy in patients with chronic B viral (HBV) hepatitis. However, several opinions argued that in patients over 40 years of the age, the treatment should be started if they have elevated serum HBV DNA levels without elevated ALT levels. Initiating the treatment in these patients may still be argued by the concept that the rate of HBeAg loss depends on the pretreatment ALT level The aim of this study was to investigate the usefulness of pretreatment ALT level in predicting HBeAg loss in patients aged over 40 years under lamivudine treatment. We retrospectively analyzed 820 HBeAg positive patients treated with lamivudine. The patients with either currently uncured hepatocellular carcinoma or with overt liver cirrhosis were excluded. Three 356 patients met the criteria and divided into two groups, ≤ 40 years of age (Group 1) and > 40 years of age (Group 2). After the analysis using the Kaplan-Meier method and log rank test, cumulative rate of HBeAg loss was not different in two groups. Multivariate modeling indicated that elevated pretreatment ALT level was a predictor of HBeAg loss in group 1 ($p<0.05$) but it failed to behave as a predictor in group 2. Therefore, antiviral therapy might not need to be deferred until ALT level rises in HBV DNA (+) patients aged over 40.

PP-434

HBV in subsaharan Africa and SE-Asia: new subtypes, unclassifiable strains, multiple double/triple recombinations, a new genotype and several puzzles

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We investigated the 350 HBV genomes from 15 locations in 8 Subsaharan countries. Except for Cameroon (18/22 genotype A), >85% of sequences from each location belonged to genotype E with a very low diversity (1.67%) throughout all locations. In contrast, genotype A strains were highly diverse (5.1%) and separated into three new subtypes (A3, A4, A5) and a triple recombination (E/D-G-A). Thus, the diversity of genotype A is higher in Africa than anywhere else, suggesting that genotype A has developed in Africa before spreading to other continents. In contrast, the low genetic diversity of genotype E is suggestive a short evolutionary history: it would take a maximum only 200 years for the strain diversity of HBV/E viruses to develop from an unknown ancestor. This would explain its conspicuous absence in the New World, despite the forced immigration of slaves from West Africa, until the early nineteenth century. However, its widespread throughout Africa seems only possible in a naive population, which is in contradiction to the distribution and genetic diversity of genotype A. Furthermore, infection during infancy is mostly associated with chronic carrier status but could hardly account for the explosive spread of virtually identical viruses in Africa. In SE-Asia, we found multiple different subtypes of B and C, mixed infections as well as numerous related new strains that fulfilled the criteria of a new HBV genotype (I) with two subtypes (I1 and I2). In Asia a high frequency (>20%) of mixed infections was found including recombinations with this new genotype.

PP-435

Activated B cells carrying HBcAg peptide may serve as antigen-presenting cells to induce HBV-specific CTL response in vitro

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Objective: To investigate whether peripheral B lymphocytes may be activated by CD40L and then loaded with HBcAg18-27 peptide, and whether such B cells may serve as antigen-producing cells (APCs).

Method: PBMCs from blood donors and hepatitis B virus infection patients were obtained by Ficoll-density centrifugation. B cells were isolated from PBMCs with anti-human CD20 immunomagnetic beads, while the remained cells served as T cells, The B cells were cultured in the presence of CD40L and IL-4 for 4~8 days, followed by a further incubation for 12 hours after adding synthetic HBcAg18-27 peptide. The T cells were co-cultured with HBcAg18-27 peptide loaded activated B Cells for 5 days. Cell cycle analysis and surface molecules on the B cells were tested by cytometry. The HBcAg18-27-specific CTLs were detected by Pro5TM MHC pentamers.

Result: Cell cycle analysis showed that, after cultured in the presence of CD40L and IL-4, the surface molecules, including MHC-1, MHC-2, CD80, and CD86, on the activated B cells were significantly up-regulated. Based on the results of flow cytometry and fluorescence microscopy, approximately 41.3% of the activated B cells were loaded with HBcAg18-27 peptide. After co-culture with activated and the peptide-loaded B cells, 2.68 ± 0.19% of the T cells were induced to be HBcAg18-27-specific CTLs, while only 0.41 ± 0.10% of the T cells were specific when the T cells were cultured in the absence of these B cells. The difference was significant ($p<0.01$).

Conclusion: Human B cells may be activated by CD40L to proliferate and differentiate. The activated B cells with loaded HBcAg18-27 peptide may serve as APCs to induce T cells to be HBcAg18-27-specific CTLs.

PP-436

Clevudine showed greater antiviral effect in HBeAG(+) chronic hepatitis B patients when compared with lamivudine

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Background: Clevudine is a pyrimidine analogue with potent anti-HBV activity in vitro. 24 week therapy with clevudine 30 mg daily has been shown to result in profound viral suppression with normalization of serum ALT levels.

Aim: To compare antiviral potency of clevudine versus lamivudine at week 48.

Methods: Twenty-six HBeAg positive treatment naïve patients with serum ALT> ULN were randomized 1:1 to receive either clevudine 30 mg daily (n=16) or lamivudine 100 mg daily (n=16) for 48 weeks. Serum was collected on Day 0, 1, 4, 7,10, 17, week 3, 4, 8, 12, 16, 24, 32, 40 and 48. HBV DNA was quantified by real-time PCR assay with a linear range of 30-10^8 copies/ml.

Results: The clevudine group demonstrated greater viral suppression on Day 7 when compared with the lamivudine group [median reduction (range) 1.8 (0.7-3.2) vs. 1.2 (0.4-2.4) $\log_{10}$ copies/ml respectively, $p=0.03$]. This greater viral suppression persisted until week 48 [median reduction (range) 4.9 (4.4-6.6) vs. 3.5 (2.5-8.5) $\log_{10}$ copies/ml respectively, $p=0.03$]. Three of the 16 patients (18.8%) in clevudine group developed HBeAg seroconversion when compared with 2 of the 16 patients (12.5%) in lamivudine group (p=NS). No patients in either group had viral breakthrough at week 24. Clevudine was well tolerated with similar adverse event with the lamivudine group (p=NS).

Conclusion: Clevudine demonstrated superior viral suppression when compared with lamivudine at week 1 therapy and this superior antiviral efficacy persisted at least to week 48 of therapy.

PP-437

Estimation of liver inflammation and necrosis in patients with chronic hepatitis B through A biochemical grading system

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Objective: To establish a new grading system to evaluate liver inflammation and necrosis in patients with chronic hepatitis B through clinical biochemical assays.

Methods: Clinical and pathological data were collected from 233 cases with chronic hepatitis B. 19 biochemical items were analysed and 5 items were selected in our grading system. Each of five items were scored 0 to 4 based on the different values. The extent of liver inflammation and necrosis was evaluated according to the total score.
Results: ALT、AST、ChE、γGT and TBA were enterd into our grading system. The grade of liver inflammation and necrosis was consided less than 2.0 with the total score lower than 6. Higher grade of liver inflammation and necrosis was considerd with higher total score. It shared an identity of 82.8% in estimation results and real grade of liver inflammation and necrosis. The identity rate in adults was significantly higher than in children (86.7% to 69.8%, p=0.004). This grading system was applied to pick the patients whose liver inflammation and necrosis was equaled to or higher than grade 2.0. It exhibited the sensitivity of 83.8%, specificity of 81.2% , positive prediction value of 88.6% , negative prediction value of 74.2% in all cases and 88.0%, 84.7%, 90.6%, 82.43% in adluts respectively.
Conclusion: Our data suggest that the grading system can evaluate the extent of liver inflammation and necrosis in patients with chronic hepatitis B. It helps to direct the clinical antiviral therapy.

PP-438
Long term efficacy, safety and resistance analyses of entecavir (ETV) treatment in Japanese nucleoside-Naive patients with chronic hepatitis B (CHB)
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Background: The aim of CHB treatment is sustained suppression of hepatitis B virus (HBV) replication. Nucleoside-naive patients who completed 24 or 52 weeks of treatment in Japanese ETV Phase II studies ETV-047 (ETV 0.01mg, 0.1mg, 0.5mg or Lamivudine 100mg) and -053 (ETV 0.1mg and 0.5mg) could enroll in an open-label rollover study (ETV-060). In these patients, the efficacy, safety and resistance profile of long-term treatment with 0.5mg ETV were evaluated.
Methods: Ninety-four percent (32/34) of ETV-047 patients and 100% (34/34) of ETV-053 patients treated with ETV 0.5mg enrolled in ETV-060 without a treatment gap. HBV DNA, histological improvement, ALT, and safety were evaluated through 3 years. HBV DNA sequencing was performed on all patients whose HBV DNA remained detectable by PCR (≥400 copies/mL).
Results: Forty-six of 66 patients were treated with ETV 0.5mg for 144–148 weeks in ETV-047,053/060. The proportion of patients achieving HBV DNA <400 copies/mL was 87% (40/46). Histological improvement was observed in 100% (19/19) of patients with repeat biopsies. ALT normalization occurred in 91% (41/45) of patients. Three patients discontinued due to adverse events through the study period. Ninety-eight percent of patients randomized to any dose of entecavir or lamivudine were monitored for resistance, and 5 patients had evidence of ETV-resistance substitutions through 148 weeks. In patients who received ETV 0.5mg from the beginning, the 3 year cumulative probability of ETV resistance was 1.7% (1/58).
Conclusions: Long-term treatment of nucleoside-naive Japanese patients with ETV 0.5 mg demonstrated potent anti-viral activity, histological improvement and a high barrier to resistance.

PP-439
Efficacy of melaleuca alternifolia concentrate (MAC) on HBV in vitro
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Background: Melaleuca Alternifolia Concentrate (MAC) is an extract of plant namely: Melaleuca artifolia; plant of Australia origin. It is known that MAC has efficacy against some kinds of virus and bacteria.
Aim: To determine the cytotoxicity and the antiviral activity of MAC against HBV, the test was done in vitro in a continuous cell line-2.2.15 cell line.
Method: The pure MAC diluted in DMEM was mixed with 2.2.15 cells to give final different concentrations and incubated at 37°C under 5% carbon dioxide for the different time course[1]. Viable cell number was counted by the dye exclusion method with MTT. Calculating the cell viability rate to get the optimal concentration and optimal contact time[2].The detection of hepatitis B surface antigen and hepatitis B e antigen (HBsAg,HbeAg,by ELISA (Abbott)) , the quantities of extracellular HBV DNA and intracellular HBV DNA（by real-time PCR） were measured at the different contact time points. Calculate the inhibition rate to determine the efficacy of MAC on HBV[3].
Rusults: According to the results of the cytotoxicity test and antiviral test, the optimal concentration of MAC was 0.005%, the optimal contact time was 3days. The cell viability rates of the concentration of 0.005% at the 3rd day was over 90%.The inhibition rate of s Ag ,e Ag, extracellular DNA and intracellular DNA at the optimal concentration and optimal contact time were 42.27%,40.43%,20.6% and 15.79%, respectively.
Conclution: MAC can inhibit the proteins secretion (s antigen and e antigen) and DNA replication of HBV .

PP-440
The insertion patterns of hepatitis B virus in genotypes B and C
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To identify the insertion patterns of hepatitis B virus (HBV) in China and to analyze the hot spots of various insertions, over 450 samples from chronically infected patients were analyzed and totally 79 full length sequences of HBV were obtained. In addition to high rate of substitutions and deletions, 14 inserting sequences were found in 8 individuals (10.13%). With only two samples containing preS insertions, most insertions were in core promoter. Therefore, additional 109 fragments covering core promoter and core were sequenced and insertions were detected in 23 samples (21.10%). Almost all insertions in coding regions were in frame, suggesting severe amino acid changes unbearable for mutants to survive. The hot spot of insertion occurred in a region covering nucleotide 1675 to 1765 in core promoter. Interestingly, besides very short insertion of 1-3bp, most insertions (3 to 342 nucleotides) were accompanied with certain deletions, implying the possible compensation for deletion in this regulatory region. The breakpoint of co-occurring deletion could be either the same as the insertion or at the position of 100-200bp downstream. Moreover, most inserted sequences are likely a segmental duplication of nearby sequence with 0-4 nucleotides between repeats. Furthermore, some insertions appeared from other genomes. In conclusion, we first time investigated the distribution and sequence patterns of HBV insertion in genotype B and C in large numbers of samples. Core promoter appeared as the hot spot of insertion. Many insertions were accompanied with deletions. Further analysis about the relation between insertion and disease progression are in progress.

PP-441
HBV genotypes (B/C) have significant influences on serum HBsAg levels in HBeAg positive chronic hepatitis B patients
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Aims: To investigate the impacts of HBV genotypes (B/C) on serum HBsAg levels in chronic hepatitis B patients and its possible pathway.

Methods: 492 patients were studied. Quantification of serum HBsAg was performed with Abbott I2000 and HBV genotypes were determined by polymerase chain reaction combined with restriction fragment length polymorphism. Replicative plasmids contained common genotype HBV genome was transiently transfected into Huh7 cells then HBsAg in supernatants and cell lysate was quantified.
Results: Serum HBsAg levels in patients infected with HBV genotype B (n=260) were higher ($p<0.001$) than those in genotype C (n=197). Multivariate regression analysis with all 492 subjects showed that HBV DNA levels (B=0.098, p=0.002), HBeAg status (B=0.594, $p<0.001$) and HBV genotype (B=-0.420, $p<0.001$) had significant influences on serum HBsAg levels. Regression analysis in HBeAg (+) patients (n=337) showed that HBV DNA (B=0.136, p=0.001), gender (B=0.441, p=0.011) and HBV genotype (B=-0.278, p=0.049) determined serum HBsAg levels, however, in HBeAg (-) patients (n=155) HBV DNA (B=0.195, p=0.018) was the only factor influenced serum HBsAg levels. HBsAg secretion efficiency was 33% in HBV genotype C transfection to that in HBV genotype B (p=0.010).
Conclusion: HBV genotypes affected serum HBsAg levels through different HBsAg secretion efficiency in HBeAg positive chronic hepatitis B patients.

PP-442

Antagonism of microRNA-122 by antimiR/ LNA-antimiR in HepG2.2.15 cells leads to the up-regulation of HBeAg

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Background: In the present study, we reported the effect of antagonism of miR-122 on the expression of corresponding HBV antigen in HepG2.2.15 cells.
Methods: Anti-miR-122 or LNA-antimiR-122 with liposome 2000 complexes transfected into HepG2.2.15 cells, respectively. After 24 h and 48 h, the levels of HBsAg and HBeAg expression in the supernate were detected with Time-resolved Immunofluorometric Assay kit (TRFIA) .
Results: (1) After 24 h of LNA-antimiR-122 transfection, the expression of HBeAg in the supernate of HepG2.2.15 cells had no significant change ($P>0.05$) , but after 48 h of transfection, the expression of HBeAg increased significantly ($P<0.05$). **(2)** After 24 h of anti-miR-122 transfection, the expression of HBeAg had no significant change ($P>0.05$), while after 48 h, the expression of HBeAg was also up-regulated prominently ($P<0.01$). **(3)** After 24 h and 48 h of anti-miR-122/ LNA-antimiR-122 transfection, HBsAg expression had no significant change ($P>0.05$).
Conclusion: These results show that antagonism of microRNA-122 in HepG2.2.15 cells leads to the up-regulation of HBeAg.

PP-443

More Application of HBV Virology Tools in Clinical Practice

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Many newer standardized techniques of HBV virology tools has greatly improved the management of chronic HBV infection such as HBV DNA quantification, genotyping, resistance, intrahepatic HBV cccDNA, HBsAg quantitative and semi-quantitative HBeAg assay.
HBV DNA It has been shown to predict the development of cirrhosis and hepatocellular carcinoma among Asian patients. New oral antiviral therapies has also emphasized on close monitoring of HBV DNA levels for the decision of treatment candidacy and responsiveness evaluation.
HBV Genotypes HBV genotypes generally reflect their geographic distribution and natural history of chronic infection .Genotypes can predict the HBeAg seroconversion with peg-interferon on genotype A patient.Genotype C associated with higher rate of HCC and more active disease.
HBV Resistance Nucleos(t)ide analogues inhibit the transcription of HBV DNA polymerase. But some are limited with high resistance. In general，direct sequencing, RFLP and Line Probe assay (INNO LiPa) are used for detection of HBV genotypic resistance, whereas quantitative PCR assay for detection of viral breakthrough.
HBV cccDNA Intrahepatic HBV cccDNA, serving as the transcriptional template, is produced by repair of double stranded DNA and is incorporated in the nucleosome as a stable minichromosome. it persist during chronic infection and reduce on antiviral therapy, it can be a marker of HBV infection progression. For the detection of cccDNA, two pair of primers (total DNA and cccDNA) on real time PCR are needed.
HBsAg and HBeAg New data suggested that quantitative HBsAg assay can serve as a tool for monitoring HBV replication on patients with IFN-a or lamivudine therapy. The required HBIG doses during liver transplantation can be adjusted in accordance with HBsAg quantification. Serum HBsAg levels are positively correlated with cccDNA levels during adefovir combined with Peg IFN-a therapy. HBeAg concentration more than 100 IU/mL at 12wks during PEG IFN-a treatment is well correlated with non-response.

PP-444

Immune responses to antiviral and immunomodulatory treatment after thymosin-alpha 1 therapy on hepatitis B patients

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Object: To evaluate the immune responses by chronic hepatitis B (CHB) patients after Thymosin-alpha 1 (TA1) therapy.
Method: Total 19 cases enrolled in Phase II clinical trial accepted TA1 therapy. A three-color flow cytometry was used to detect cytokines secreted by both of Th1 cell and Th2 cell secretion before treatment, week 13, 21, 37, 52 and the serum HBV DNA level, liver function and other serum markers were detected at the same time.
Results: In all patients treated with TA1, the cytokines secreted by both Th1 and Th2 were significantly increased and significantly higher than that in control group ($p<0.05$). All of the cytokines were increased from 13 weeks to 37 weeks continuously, excepted for IL-2 and IL-6, which increased incessantly from 13 weeks to 21weeks, but maintained from 21 weeks until 37 weeks. While from 37 weeks to 52 weeks, the cytokines productivities decreased except for IFN, IL-4 and IL-6. However, there was no relevance among the levels of cytokines, ALT level and HBV DNA.
Conclusion：The results showed that immune regulator with TA1 can increase the Th1/Th2 cytokine secretion in the patients with chronic hepatitis B. The cytokines secreted by Th1 and Th2 cells were not associated with AST, ALT and HBV DNA levels.

PP-445

The clinical effect of IFN-α2a with oxymatrine on chronic viral hepatitis B

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Objective: To investigate the clinical effect of IFN-α2a with oxymatrine on chronic viral hepatitis B and to look for new methods for treating hepatitis B
Methods: randmized study was used, In this study; 149 patients were allocated to INF-α2a, Oxymatrine, IFN -α2a with oxymatrine and glucose groups to –observe ALT, AST and viral marker change
Results: At the end of treatment, the rate of normal ALT , the negative rate of HBV-DNA and HBeAg, and the positive rate of HBeAb were similar in oxymatrine .IFN-α2a ,IFN –α2a with oxymatrine group,It was highter than that of glucose group .After 12 months follow up , the totol effective rate is 32%,41.6%,58.6%. in oxymatrine .IFN-a 2a,IFN-α2a with oxymatrine groups, respectively.
Conclusions: IFN-α2a with oxymatrine effective to treat hepatitis B with a good negative rate of HBV-DNA and positive rate of HBeAb.

PP-446

Hepatitis B infection in Chinese immigrants of New York City

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Context: The burden of HBV-infection is disproportionately high in Asian Americans in the U.S., particularly those born in China where HBV is highly-endemic.
Objective: To analyze epidemiologic characteristics of HBV-infection in Chinese communities in NYC and examine factors that affect prevalence rates in this population.
Methods: Retrospective analysis of 1955 participants who were screened for the first time for the presence of HBsAg and HBsAb in a community-based city-wide HBV screening program for Asian Americans in NYC, 2005-2007.
Results: The overall prevalence of HBsAg was 22.5% (95%CI: 20.7-24.4). Participants testing HBsAg+ were more likely to be men

(POR=2.1; 95%CI:1.6-2.7), age below 40 (POR range 4.1-4.6 compared to age >60 years), to have been born in Fujian province (POR=3.1; 95%CI: 1.4-6.7 compared to Hong Kong) and to have a family member who is HBV-infected (POR=1.5; 95%CI: 1.1-2.1). Rates of HBsAg seroprevalence did not differ by length of residence in the U.S., but varied by specific province/region of birth.

Conclusions: Chronic HBV infection is a major health disparity among Chinese immigrants in NYC. The rate of infection varied greatly by gender, age, ifamily member infection and region of birth. Knowledge of these factors may provide better estimates of the burden of infection among Chinese immigrants and better allocation of resources to prevent new infections and disease progression in those already infected. Understanding the basis of the difference in seroprevalence between the provinces may be useful in developing strategies to decrease the rate of HBV-infection within China and, thereby, its impact globally.

PP-447

Characteristics of hepatitis B patients commonly encountered in Asia

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Background: Chronic hepatitis B (CHB) is highly prevalent in Asia. The present study is a survey of disease characteristics and patient management that aims to characterise the types of CHB patients encountered in Asia.

Methods: An analysis was conducted of a patient records database containing data collected from a survey between February and May 2006.

Results: Medical records of 2499 CHB patients from China (1499), Korea (700), and Taiwan (300) were included. The proportions of HBeAg-positive and -negative patients were approximately equal (1018 and 1437 patients, respectively). Fewer negative patients (65%) were undergoing treatment compared with positive patients (89%). At treatment initiation, 73% of HBeAg-positive patients had HBV DNA <9 $\log_{10}$ and ALT ≥ 2 x ULN while most of HBeAg negative had HBV DNA <7 $\log_{10}$ (80%). 22% of positive patients were not on therapy; 35% had HBV DNA ≤ 5 $\log_{10}$ and 88% had ALT<2 x ULN at their latest visit. 62% of untreated negative patients had HBV DNA ≤ 5 $\log_{10}$ and 100% had ALT <2 x ULN. In untreated patients for which the liver status was reported, 88% positive and 91% negative patients had no or mild liver fibrosis.

Conclusions: Most CHB patients in Asia have ALT ≥ 2 x ULN and significant liver disease at treatment initiation. HBV DNA levels at the start of treatment were <9 $\log_{10}$ and <7 $\log_{10}$ in a large proportion of positive and negative patients, respectively. Patient profiles were consistent in China, Korea, and Taiwan.

PP-448

Mutations in basal core promoter and precore regions of HBV and their association with disease status

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Aim: To investigate the distribution of BCP and precore mutations in HBV and possible correlation of these mutations to clinical conditions.

Methods: DNAs from 156 patients of northern China were purified. Target regions were amplified followed by sequence analysis.

Results: 131 of 156 samples contained BCP and precore mutations. Substitutions at 1726, 1727, and 1730, which were not reported previously, were frequently detected and more than one such mutation were often observed in single sequence. Interestingly, combinations of these substitutions with the known double mutation A1762T/G1764A appeared to significantly correlate with the positivity of anti-HBe (*P<0.001*). Other substitution combinations, including A1762T, G1764A, and G1896A, as well as single mutations of C1776T/G, A1846T, G1864A, and G1896A were also often seen in anti-HBe positive samples (*P<0.05*). However, the presence of G1776A, A1846T, G1857A, and G1896A were significantly associated with HBeAg-negativity (*P<0.01*). Although most mutations in TA region were located in TA1 or TA2, they appeared not to associate with HBeAg/anti-HBe status. Analysis with clinical symptoms revealed correlation of T1752C/A, A1762T, G1764A, and G1896A with severer conditions (*P<0.01*). In most male patients, the presence of double mutation A1762T/G1764A occurred commonly which was also correlated with disease progression (*P<0.01*). More combinations of point mutations, including triple mutation T1753C, A1762T, and G1764A, seemed significantly to associate with CSH, LC, and HCC (*P<0.05*).

Conclusion: Many BCP and precore mutations were associated with the appearance of HBeAg and anti-HBe, and the severity of liver diseases. The combination of single mutations may also play roles in disease progression.

PP-449

Comparisons of virological characteristics between inactive HBsAg carriers and HBeAg negative chronic hepatitis B patients in Guangdong province, China

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Aims: To compare the possible differences of viral characteristics between inactive HBsAg carriers (HBsAg-IaC) and HBeAg negative chronic hepatitis B patients (e^--CHB).

Methods: HBsAg-IaC (n=187) and e^--CHB (n=99) were consecutively collected and studied. Mutations at nt1896 and nt1762/1764 were analyzed by direct sequencing (nt1600-1974), and HBV genotypes were determined with direct sequencing in combination with polymerase chain reaction-restriction fragment length polymorphism assay. Serum HBsAg quantification was performed on Abbott I2000 with Architect HBsAg assay.

Results: Serum HBsAg levels were not significantly different between these two groups (p=0.432). 113 HBsAg-IaC had low level viraemia detected by nested-PCR and genotypes then were determined, and direct sequencing was successfully performed in 103 cases. The prevalence of HBV genotype B (HBV/B) infection (69/103 vs 39/99, p<0.001) and G1896A mutation (69/103 vs 39/99, p<0.001) was higher in HBsAg-IaC than in e^--CHB, however the prevalence of A1762T/G1764A mutations was lower in former group than in latter one (38/103 vs 65/99, p<0.001). Male (OR=7.681 , 95%CI=2.693-20.992, p<0.001), older than 40 (OR=24.421, 95%CI=5.187-114.969, p<0.001), HBV genotype C (HBV/C) infection (OR=2.695, 95%CI=1.240-5.859, p=0.012) and occurrence of A1762T/G1764A mutations (OR=2.116, 95%CI=1.012-4.425, p=0.046) were associated with e^--CHB.

Conclusions: Viral characteristics, including HBV genotypes distribution, occurrences of mutations as G1896A and A1762T/G1764A were different between HBsAg-IaC and e^--CHB. And the male and older HBsAg-IaC infected with HBV/C and harbored A1762T/G1764A mutations should prompt regular follow-up.

PP-450

Efficacy of melaleuca alternifolia concentrate (MAC) on HBV in vitro

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Background: Melaleuca Alternifolia Concentrate (MAC) is an extract of plant namely: Melaleuca artifolia; plant of Australia origin. It is known that MAC has efficacy against some kinds of virus and bacteria.

Aim: To determine the cytotoxicity and the antiviral activity of MAC against HBV, the test was done in vitro in a continuous cell line-2.2.15 cell line.

Method: The pure MAC diluted in DMEM was mixed with 2.2.15 cells to give final different concentrations and incubated at 37°C under 5% carbon dioxide for the different time course[1]. Viable cell number was counted by the dye exclusion method with MTT. Calculating the cell viability rate to get the optimal concentration and optimal contact time[2].The detection of hepatitis B surface antigen and hepatitis B e antigen (HBsAg,HbeAg,by ELISA (Abbott)) , the quantities of extracellular HBV DNA and intracellular HBV DNA（by real-time PCR） were measured at the different contact time points. Calculate the inhibition rate to determine the efficacy of MAC on HBV[3].

Results: According to the results of the cytotoxicity test and antiviral test, the optimal concentration of MAC was 0.005%, the optimal contact time was 3days. The cell viability rates of the concentration of 0.005% at the 3rd day was over 90%.The inhibition rate of s Ag ,e Ag, extracellular DNA and intracellular DNA at the optimal concentration and optimal contact time were 42.27%,40.43%,20.6% and 15.79%, respectively.

Conclusion: MAC can inhibit the proteins secretion (s antigen and e antigen) and DNA replication of HBV.

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PP-451

The adefovir-lamivudine combination therapy effectively suppresses the emergence of genotypic resistance to adefovir in lamivudine resistant hepatitis B

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Background and aims: There are increasing data on ADV resistance in lamivudine (LAM) resistant CHB. This study aimed to characterize the biochemical and virological response of ADV monotherapy and ADV-LAM combination therapy for treatment of LAM-resistant CHB.

Methods: Forty one patients with ADV-LAM therapy (combination group) and 82 age, sex-matched patients with ADV monotherapy (monotherapy group) were followed-up in Kyungpook National University Hospital due to biochemical breakthrough from genotypic resistance to LAM. Liver function test, HBe antigen/antibody titer and HBV DNA levels were evaluated before and every 3 months after ADV therapy.

Results: 1) The patients with biochemical response at 48 week were 28 (68.3%) in combination group and 65 (79.3%) in monotherapy group (p=0.18). 2) The patients with virologic response at 48 weeks were 16 (39.0%) in combination group and 37 (45.1%) in monotherapy group (p=0.728). 3) Loss of HBe antigen at 48 week was observed in 4 patients (9.8%) in combination group and 7 patient (8.5%) in monotherapy group (p=1.00). 4) The mean serum HBV DNA level was 4.639□1.4134 Log10/ml in combination group and 4.856□1.5172 Log10/ml in monotherapy group (p=0.56). 5) Genotypic resistance to ADV was detected in 5 patients in monotherapy group, while no genotypic resistance in combination group. 6) The rtA181V mutation occurred in 3 patients, rtN236T mutation in one patient, and rtA181V + rtN236T double mutation in one patient.

Conclusions: There were no significant differences in serum aminotrasferase and HBV DNA levels between twp groups, but ADV-LAM combination therapy effectively suppressed genotypic resistance to ADV.

PP-452

One year of Entecavir therapy for Chinese patients with hepatitis B e antigen positive chronic hepatitis B

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Objective: To evaluate the efficacy and safety of entecavir treatment in patients with hepatitis B e antigen (HBeAg)-positive chronic hepatitis B who had not previously received a nucleoside analogue.

Methods: Sixty-two patients received 48-week entecavir 0.5mg/d therapy. Serum HBV DNA load was measured with quantitative real-time-PCR. Alanine aminotransferase (ALT) activity, HBeAg, anti-HBe-antibodies, HBV DNA level in serum were evaluated at baseline, week 2, 12, 24 and 48 during therapy. Evaluation of safety and tolerance was based on clinical adverse events and laboratory analyses.

Results: HBV DNA levels declined sharply by around 3 $\log_{10}$ copies/ml during the first two weeks, with a highly significant reduction ($p<0.0001$) at week 2 and thereafter, as compared to those at baseline; 37%, 57% and 80% of the patients had undetectable serum HBV DNA levels at week 12, 24 and 48 respectively. Highly significantly decreasing serum ALT ($p<0.0001$) occurred during the first 2 weeks of the study. At week 48, ALT levels were normalized in 84% of the patients. HBeAg seroconversion (HBeAg negative, HBeAb positive) was achieved in 8.1% and 16.1% of patients by 24 and 48 week. At the end of 24th and 48th weeks, complete response (ALT normalization and HBV DNA and HBeAg loss) was observed in 11% and 16%, respectively. There was no evidence of drug resistance or adverse effect in CHB patients treated for up to 48 weeks.

Conclusion: Entecavir was well tolerated and resulted in virological and biochemical improvements in HBeAg positive chronic hepatitis B patients.

PP-453

Effect of e antigen status and age on chronic hepatitis B

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Purpose: About 10 million population has been suffering from chronic hepatitis B(CHB) in Bangladesh. E antigen negative CHB is prevalent all over the world. Age is positively correlated with advancement of CHB. The present study was designed to verify the effect of age and e antigen status on CHB.

Methods: This study was done during the period of March 2004 to October 2007. We have included 2617 CHB patients. Other causes of CHB were excluded. Chi square test, independent t test and General Linear Model (GLM) type I and III were done whenever required.

Results: Mean age of the study population were 28.9 ± 13.7 years. Out of these 1039 (39.7%) were e antigen negative, 242 (9.2%) were cirrhotic. Male: female was 8.9: 1. E antigen negatives were more older ($p<0.001$), and fibrotic ($p= 0.026$) and contained less DNA load ($p< .001$), ALT and AST level ($p< .001$). Histological activity did not significantly differ in relation to e antigen status. If we analyze with GLM e antigen had no significant effect on fibrosis after removing the effect age rather age had significant effect on fibrosis ($p<.001$), DNA load, ($p<0.05$) even after removing the effect of e antigen.

Conclusion: E antigen less significantly interferes with different character of CHB. Advancement of CHB and e antigen negativity both are consequences of older age (longer duration of infection) in the natural course of CHB.

PP-454

Pegylated interferon alfa-2a alone or in combination with lamivudine for HBeAg-negative chronic hepatitis B: Multicenter Study

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Aim: To compare the efficacy of a combination of pegylated interferon-alfa2a (PEG-INF) and lamivudine (LAM) with PEG-INF alone in the treatment of patients with HBeAg negative chronic hepatitis B (CHB).

Methods: 36 HBeAg-negative patients with chronic hepatitis B were assigned combination therapy (180 microg/week pegylated interferon alfa-2a and 100 mg/day lamivudine) or monotherapy (180 microg/week pegylated interferon alfa-2a) for 48 weeks. All included patients were followed up for 24 weeks after treatment. All patients were infected with hepatitis B virus (HBV) genotype D. A post-treatment response was defined as alanine aminotransferase (ALT) normalisation and nondetectable hepatitis B virus (HBV) DNA level.

Results: Thirty-six hepatitis B virus (HBV) E antigen-negative chronic hepatitis B patients received pegylated interferon alfa-2a either alone or with lamivudine for 48 weeks and were followed for an additional 24 weeks. In both treatment arms, peginterferon alpha-2a (with or without lamivudine) therapy, younger age, female gender, high baseline ALT and low baseline HBV DNA were identified as significant predictors of combined response at 24 weeks post-treatment. At the end of follow-up, virological response rates (HBV DNA levels of <400 copies/ml) were similar in the monotherapy (35%) and combination therapy (37%) groups.

Conclusion: Treatment with pegylated interferon alfa-2a is effective for HBeAg-negative chronic hepatitis B. Combination with lamivudine in the regimen used is not superior to monotherapy.

PP-455

Pegylated interferon alfa-2a alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: Multicenter Study

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Aim: To compare the efficacy of a combination of pegylated interferon-alfa2a (PEG-INF) and lamivudine (LAM) with PEG-INF alone in the treatment of patients with HBeAg positive chronic hepatitis B (CHB).

Methods: 30 HBeAg-positive patients with chronic hepatitis B were assigned combination therapy (180 microg/week pegylated interferon alfa-2a and 100 mg/day lamivudine, n=12) or monotherapy (180 microg/week pegylated interferon alfa-2a, n=18) for 48 weeks. All included patients were followed up for 24 weeks after treatment. All patients were infected with hepatitis B virus (HBV) genotype D. A post-treatment response was defined as alanine aminotransferase (ALT) normalisation and nondetectable hepatitis B virus (HBV) DNA level.

Results: In logistic regression analyses across both treatment arms, peginterferon alpha-2a (with or without lamivudine) therapy, younger age, female gender, high baseline ALT and low baseline HBV DNA were identified as significant predictors of combined response at 24 weeks post-treatment. At the end of follow-up, virological response rates (HBV DNA levels of <400 copies/ml) were similar in the monotherapy (39%) and combination therapy (42%) groups (p=0.66). Three (28%) of 18 patients assigned monotherapy and four (33%) of 12 assigned combination therapy had lost HBeAg at the end of follow-up (p=0.78). Patterns were similar when response was assessed by suppression of serum hepatitis B virus (HBV) DNA or change in concentrations of alanine aminotransferase.

Conclusion: Treatment with pegylated interferon alfa-2a is effective for HBeAg-positive chronic hepatitis B. Combination with lamivudine in the regimen used is not superior to monotherapy.

PP-456

Regulatory polymorphisms in the promoter of CXCL10 Gene and disease progression in male hepatitis B virus carriers

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In this report we investigated whether the naturally occurred sequence variations in the *CXCL10* gene impact liver damage and disease progression of chronic hepatitis B virus (HBV) infection. We systematically screened sequence variations in the *CXCL10* gene, and examined the association between the variations in this gene and susceptibility to the disease progression of chronic HBV infection in 2400 Chinese from Beijing and Chongqing. We identified the polymorphism G-201A, was associated with the susceptibility to disease progression in male HBV carriers (odds ratio = 1.53, $P = 0.001$). Functional analyses showed that the G-201A polymorphism alters the binding affinity of nuclear protein and regulates the *CXCL10* expression. We observed higher *CXCL10* transcription in interferon-γ stimulated peripheral blood mononuclear cells with the disease-susceptible genotypes and augmented CXCL10 production in serum and liver tissues of progressed HBV carriers. The novel regulatory polymorphism G-210A in the promoter of *CXCL10* gene could be a part of the genetic variation underlying the individuals' susceptibility to disease progression of chronic HBV infection.

PP-457

Elevated expression of inducible nitric oxide synthase in chronic hepatitis B patients: correlated with histopathological grading and staging

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Objective: To investigate the intrahepatic expression of inducible nitric oxide synthase(iNOS) in patients with chronic hepatitis B(CHB) and its relation to liver histopathology．

Methods: The intensity and distribution of the immunohistochemical staining of intrahepatic iNOS were studied in the liver biopsies obtained from seventy-four patients with CHB and statistical analyses were performed between intrahepatic iNOS and ALT，HBeAg，HBV DNA，grading of liver inflammation and staging of fibrosis．Seven histologically normal liver sections were used as a control group．

Results: Compared with the control group，the intrahepatic iNOS immunoexpression was significantly higher in patients with CHB($P<0.05$)．iNOS immunoreactivity was observed mainly in hepatocytes，showing a predominant cytoplasmic staining，with the positive liver cells distributed diffusely throughout the hepatic lobule．Immunopositive staining could also be detected in Kupffer cells，sinusoidal lining cells and vascular endothelial cells．Compared with patients with normal ALT，the hepatocelluar iNOS immunoexpression was significantly higher in patients with elevated ALT($P<0.05$) and the iNOS immunoexpression was significantly correlated with the serum level of ALT($r=0.601, P=0.000$)．Statistical analysis also showed that the intrahepatic iNOS immunoexpression was positively correlated to the grading of liver inflammation and the staging of liver fibrosis($r=0.660, P=0.000$；$r=0.507, P=0.000$)．No significant correlation between iNOS and HBeAg and HBV DNA was detected．

Conclusions: Our results showed that the intrahepatic expression of iNOS is elevated in chronic hepatitis B patients and correlated well with the severity of the disease，which indicated that inducible nitric oxide synthase may have a critical role in the pathogenesis of chronic virus hepatitis．

PP-458

Analysis of 27 cases patients with chronic severe hepatitis B associated with lamivudine -resistant mutation

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Objective: To study characteristics of chronic severe hepatitis B associated with drug-resistant mutation during lamivudine treatment.

Methods: Retrospective analysis of 27 patients who exhibited chronic severe hepatitis B associated with drug-resistant mutation during lamivudine treatment. YMDD motif mutation was detected in those patients by the gene chips or DNA sequencing. The pathological features of liver after liver transplantation from 8 patients were analyzed.

Results: Among the 27 patients with chronic severe hepatitis B associated with lamivudine-resistant mutation, 8 patients healed, 8 patients had liver transplantation, 11 patients died. The reasons of death were: hepatic encephalopathy (4 cases), upper peptic tract hemorrhage (3 cases), hepatorenal syndrome (3cases) and severe infection (1cases). Compared to cirrhotic group, prevalence of severe hepatitis of noncirrhotic group was lower, the age of patients was younger, the outcome was more favorable. The pathological features of liver after liver transplantation were two types as follows: active hepatic cirrhosis and massive or submassive hepatic necrosis.

Conclusion: The outcome of chronic severe hepatitis B associated with lamivudine-resistant mutation was poor, hepatic cirrhosis was high risk factor of chronic severe hepatitis B associated with lamivudine-resistant mutation. There may be two kind of mechanism of chronic severe hepatitis B associated with lamivudine-resistant mutation: one was hepatic decompensation on the basis of hepatic cirrhosis, another was intense immune response leading to massive or submassive hepatic necrosis.

PP-459

Polymorphism of HLA-DRB1 of the uyghur in chronic hepatisis B in Khotan Area, Xinjiang, China

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Objective: To understand the polymorphism of HLA-DRB1 of the Uyghurs in Khotan Area，Xinjiang，China and the association with chronic hepatisis B. Methods Reverse-polymerase chain reaction sequence specific oligonucleotide probes（PCR- SSOP）combined with Luminex Flow Cytometry were used to genotype HLA-DRB1 alleles in 56 unrelated healthy Uyghurs controls and 41 chronic hepatisis B Uyghurs patients of Khotan Area, Xinjiang, China.

Results: 13 DRB1 alleles covered all serology specificities could be detected，and their frequencies were calculated in two groups.

Conclusio: This study provided the normal allele frequencies of HLA-DRB1 and linkage disequilibrium with chronic hepatisis B in Uyghurs in Khotan Area，Xinjiang of China, which may be of significance in the studies on population genetics and disease association.

PP-460

The preliminary study of the correlation between quasispecies and liver biochemical changes in chronic HBV infection during immune clearance period

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To study the relationship between quasispecies and liver biochemical changes in chronic HBV infection immune clearance period. A total of 104 CHB patients in immune clearance period who were treated with LAM were studied. HBV DAN and ALT of 12 、24 、36 weeks patients' serum were detected by quantitative PCR and biochemical analyzer.After 24 weeks treated with LAM, contrary to all patients who HBV DNA less than detection limit (<1000 copy / ml) recoveried normal ALT, none of patients who virology no response to anti-virus treatment can resume normal ALT, and some of patients who partial response to anti-virus treatment resumed normal ALT. There is a significant difference between three response groups ($p<0.05$). Not all but part virus strains of HBV Quasispecies induce host immune system response causing ALT changes and Immune system maintains tolerance to other HBV virus strains.

PP-461

Hepatitis B prevalence in Ahwaz blood donors during 2005-2007

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Of the 2 billion people who have been infected with the hepatitis B virus (HBV), more than 350 million chronically infected persons are at high risk of death from cirrhosis of the liver and liver cancer, diseases that kill about one million persons each year. Because the high number of blood dependent patients in our city, study of HBV prevalence helps us in evaluating the efficacy of our program in reduce the HBV infection.

In this descriptive survey we studied HBV prevalence in 119075 blood donors that referred to Ahwaz blood transfusion center for 30 months. Reactive samples in screening for HBS-Ag by using Enzyme Linked Immuno Sorbent. Assay (ELISA) duplicate retested and repeated reactive samples confirmed by HBC-Ab and neutralization test. Data were analyzed in SPSS 11.5 by using chi square test.

We found (99/47254) in first year, (83/44535) in second year and (47/27286) and we found decrease in HBV prevalence. We found significant difference between HBV infection age and marital status. Female blood donors showed low BV infection.

HBV prevalence was less than other regions in our country. Our findings showed decreasing in HBV infection, because public HBV vaccination, educational program, self exclusion unit and software program for blood donor's recruitment. Focus to low risk blood donors provide safe blood transfusion and grantees blood safety.

PP-462

Tea polyphenols exerts anti-hepatitis B virus effects in a stable HBV-transfected cell line

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A variety of research studies revealed that Hepatitis B virus (HBV) is the major cause of end stage liver diseases and hepatocellular carcinoma. Now therapeutic strategies can effectively inhibit proliferation of HBV. However, development of drug resistance is a major limitation to their long-term effectiveness. In order to improve this condition, in this study we investigated the anti-HBV effects of tea polyphenols. we measured the quantity of HBsAg, HBeAg and HBV-DNA released in the supernatant of the cultured HepG 2.2.15 cells after drugs of various concentration exposure for 3,6,9 days. Tea polyphenols displayed some inhibitory action on the secretion of HBsAg and exerted significantly inhibition of HBeAg secretion dose-dependently ($p < 0.01$), but the time-dependent function is not so significant. The HBV-DNA expression in the supernatant of the cell culture also decreased markedly in a dose-dependent ($p < 0.01$) way. Tea polyphenols possessed potentially anti-HBV effects, and it could be considered as an alternative therapeutic agent to manage the HBV infection.

PP-463

Immune reconstitution after adefovir dipivoxil therapy in patients with chronic hepatitis B

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Objective: To evaluate the immune reconstitution by chronic hepatitis B (CHB) patients after adefovir dipivoxil therapy.

Method: A treatment was given to 22 CHB patients who were enrolled in Phase IV clinical trial and 10 healthy subjects were used as a control. Two classes of cytokines including IL-2, IFN-γ, TNF-β, IL-4, IL-6 and IL-10 were measured using a three-color flow cytometry before treatment and at 12, 24, 36 and 52 weeks after treatment. Samples were also tested for HBV DNA, ALT and AST.

Results: Before treatment, the cytokine production by PMBC from CHB patients was about 6 times lower for IFN-r, TNF-a, IL-4 and IL-6 and 15 times lower for IL-2 and IL-10 than the controls. Along with treatment all the cytokines production were continuously increased from 12 weeks to 24 weeks, except for IL-2 that increased continuously until 36 weeks. While from 36 weeks to 52 weeks the cytokines productivities decreased except for IL-4 and IL-6. The cytokines secreted by Th1 and Th2 cells were not associated with ALT and HBV DNA levels, but was independently related with AST level.

Conclusion : The increased cytokine production showed that the treatment induced immune responses involving both Th1 and Th2 cytokines, which would be important for viral clearance. Thus, the change in cytokines production can be a sign of the effectiveness of the antiviral treatment and could be used as clinical indices.

PP-464

Characteristics of HBV quasispecies in uighur patients with chronic hepatitis B virus infection in Xinjiang

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Aim: To study characteristic of hepatitis B virus (HBV) quasispecies in Uighur patients with chronic hepatitis B infection in Xinjiang.

Methods: We choose five Uighur patients with chronic HBV infection, including chronic hepatitis B and liver cirrhosis, and one of those is acute exacerbation on chronic hepatitis B. We determine quasispecies of HBV reverse transcriptase (HBV RT) region by using PCR-Clone-sequence analysis. HBV RT region was amplified and cloned by nested PCR and TA cloning. 33 clones from a single sample was measured by sequence analysis to determine the different clonetypes.

Results: The complexity and heterogeneity of quasispecies of this region were 12 and 14 with lamivudine treatment patients,and were 7,8,10 and 5 without lamivudine treatment patients. The complexity and heterogeneity of quasispecies of this region were low (8)at the time points of exacerbation of chronic hepatitis and high (10) at the time points of recovery of chronic hepatitis. The main style of variant is point mutation. V173,L180,A181,A184,S202,M204,N236,M250 mutations have not been detected in RT gene in the predominant clones of HBV strain .rtA222T mutation has been detected in RT gene in the predominant clones of HBV strain that can not be detected usually.

Conclusion: There does exist quasispecies phenomenon in HBV RT region in Uighur CHB patients in Xinjiang. It is possible that the shift of quasispecies of HBV RT region is related with antiviral treatment and clinical courses. The complexity and heterogeneity of quasispecies of RT region may be unity without under drug pressure.

PP-465

Combination anti-hepatitis B virus effects of 2', 3'-dideoxy 3'fluoroguanosine with nucleos(t)ide Analogs

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Introduction: 2', 3'-dideoxy-3'fluoroguanosine (FLG) is a nucleoside analogue which acts as an inhibitor of viral DNA polymerase and a chain terminator of viral reverse transcription. FLG has been shown to be active against wild-type and drug-resistant HBV in the in vitro models. In this study, we investigated the in vitro combination antiviral efficacies of FLG with lamivudine, clevudine, adefovir, tenofovir and entecavir.

Methods: Using the HepAD38 cell line as the in vitro cell model, we assayed the antiviral activities and cytotoxities of each drug alone and

in combination with FLG (Tibotec Pharmaceuticals Ltd). Real time PCR was used for the measurement of HBV DNA generated by HepAD38, and the cytotoxicity was determined by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. Using the MacSynergy II program, the combination data were analyzed through the Bliss independence model which is defined by the equation Exy= Ex+Ey-(ExEy).

Results: Analysis using the Bliss independence model indicated that FLG exerted significant synergistic antiviral effects when combined with lamivudine (V=41.51 μM^2) or tenofovir (V=95.07μM^2) and additive effects when combined with adefovir (V<25 μM^2). However, minor antagonism effects were displayed when FLG combined with entecavir (V=-32.12 μM^2) or clevudine (V=-60.25 μM^2). There was no evidence of cytotoxicity with any of the drugs when used alone or in combination at the tested doses.

Conclusions: FLG exerted different kinds of combination antiviral effects when it was used with other nucleos(t)ide analogs. Future clinical study on FLG-related combination therapy is warranted.

PP-466

In vitro anti-hepatitis B virus activities of silver nanoparticles

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Introduction: We previously reported that silver nanoparticles exhibited promising cytoprotective activities toward HIV infected T-cells. However, the effects of silver nanoparticles toward other kinds of viruses remain largely unexplored. The aim of the present study was to investigate the effects of silver nanoparticles on HBV.

Methods: HepAD38 cell line was used as the in vitro cellular model for this antiviral study of silver nanoparticles(Ag10Ns and Ag50Ns). The cytotoxicity was determined by MTT assay. Real time PCR and RT-PCR were used to quantify HBV DNA, cccDNA and RNA in HepAD38 cells.

Results: Our results revealed that the anti-HBV effects of silver nanoparticles were significant and specific compared with gold nanoparticles and other silver compounds. Ag10Ns inhibit HBV DNA replication dose-dependently after 48 h incubation. However, Ag10Ns was found to achieve maximum inhibitory effect at 5 μM level with prolonged incubation (96 h), where the anti-HBV effect was reduced at higher concentrations. Silver nanoparticles were able to inhibit the formation of intracellular HBV RNA by 72% but had little effect on the amount of HBV cccDNA.

Conclusions Silver nanoparticles could inhibit the in vitro production of HBV RNA and extracellular virions.

PP-467

Association between polymorphism of the microsomal triglyceride transfer protein (MTP) gene and HBV infection clinical outcome

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Objective: To determine whether -493 T/G and H297Q G/C polymorphisms of microsomal triglyceride transfer protein (MTP) gene were assosiated with outcomes of hepatitis B(HBV) infection.

Methods: A total of 308 HBV chronic infected subjects and 253 HBV self limited infected subjects were enrolled and examined by real-time PCR.

Results: The frequency of MTP-493 T and MTP H297Q G alleles in self-limited individuals were significantly higher than in chronic infected individuals（OR=0.13，P=0.014，95%CI 0.026-0.666；OR=0.53，P=0.048，95%CI 0.285-0.995,respectively）. The frequency of MTP H297Q CC genotype in chronic infected individuals was significantly higher than in self-limited individuals（12.66% vs 7.51%。P=0.042）.Multiple logistic regression analyses indicated an increased risk of chronic infection associated with MTP H297Q C（OR=1.32，P=0.048） after gender and age adjusted. **Conclusion**: MTP gene polymorphism is probably an important risk factor of the influence of clinical outcome of HBV infection

Poster Session – HCV

PP-468

Presence of DNA sequences, identical to hepatitis C virus, in the DNA of infected patients.

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Evidence shows that essential mixed cryoglobulinemia (EMC), B-cell-non-Hodgkin-lymphoma (NHL) and hepatoma are associated with Hepatitis C Virus (HCV) infections . HCV is an RNA virus which differs from retroviruses and possesses no integration potential. However, because of its oncogenic potential, we tried to detect its presence in the genome of mononuclear cells (MNC) of either HCV (+) patients with EMC (n=16) or NHL (n=3) and in hepatocytes of HCV (+) patients (n=42).

Methods: DNA was isolated from mononuclear cells or heptocytes of the HCV (+) patients and controls (n=22). Preliminary screening studies for detection of integration were carried out by PCR and seminested PCR processes. Positive results were further investigated by means of Southern analysis of patient's DNA. as well as probe hybridization and sequencing of PCR products of patient's DNA.

Results: Two of the EMC group and 3 of the HCV(+) hepatitis were found positive demonstrating presence of HCV sequences in their genome.

Conclusion: HCV is a virus without a known DNA intermediate stage of replication. As much as we are aware this is the first demonstration of the possible integration of HCV sequences into the DNA of MNC and hepatocytes of HCV (+) patients.

PP-469

Mutation in lymphotoxin alpha gene may increase susceptibility to hepatitis C but not schistosomal infection in Egyptian patients.

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Schistosomiasis is chronic endemic disease in Egypt that can produce portal hypertension and hepatic fibrosis. Most of complicated schistosomal cases in Egypt are co-infected with HCV. Different explanations have been proposed for the high incidence of co-infection, these include; parental schistosomal therapy and poor infection control standards during operative procedures. Different studies have illustrated a genetic predisposition for different parasitic infestation. LTα has been shown to participate in inflammatory responses and recently a single nucleotide polymorphisms in the human LTα gene can have profound effects on individual susceptibility to different diseases including parasitic infestation.

Aims: The current study aimed at investigating whether mutation in the LTα gene was associated with schistosomal and or HCV infection in a cohort of Egyptian patients and a healthy control group. Polymorphism in LTα gene was determined using PCR-RFLP.

Results: Patients with isolated HCV infection and those co-infected with schistosoma and HCV showed a significantly higher percentage of homozygote and heterozygote mutants respectively mutants compared to the control subjects ($P<0.05$), while patients with SHF did not show a significant difference compared to control group.

Conclusion: We suggest a possible role of LTα mutation in the progression of HCV infection and development of complications while LT alpha mutation is unlikely to affect susceptibility to schistosomal infection.

PP-470

Flat-dose ribavirin plus peginterferon therapy for 16 weeks may be enough in genotype 2 chronic hepatitis C patients with rapid virologic response

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Background/Aims: Standard therapy for chronic hepatitis C patients infected with HCV genotype-2 is the combination of pegylated interferon alfa and ribavirin for 24 weeks. Whether shorter treatment duration with flat-dose ribavirin plus peginterfron is possible for patients showing rapid virologic response (RVR) without compromising sustained virologic response (SVR) is unclear.

Methods: 95 Korean patients chronically infected with HCV genotype-2 were treated with peginterferon alfa-2 (2a: 180 mcg/wk, 2b: 1.5 mcg/kg/wk) plus ribavirin 800 mg/d for 12□24 weeks and followed up for 24 weeks. HCV RNA was qualitatively assessed after 4 weeks of treatment, and RVR was defined as undetectable HCV RNA(<50IU/mL) at weeks 4. Retrospectively, 57 patients were treated with standard treatment strategy (≧ 20 Weeks), 27 patients with short-term treatment strategy (≦ 16 weeks).

Results: Among the 95 patients, 84 patients (88.4%) had RVR and 92 patients (92.6%) had a sustained virologic response (SVR). All of 11 patients without RVR were treated standard treatment strategy, in whom 8 patients (72.7%) had SVR. Among the 84 patients with RVR, 27 patients were treated with short-term treatment and 57 with standard treatment. SVR was obtained in 25 of 27 patients(92.6%) in the short-term treatment group and 55 of 57 (96.5%) in the standard treatment group ($P<0.05$).

Conclusion: This finding suggests that patients infected with Korean HCV genotype-2 who have RVR can be treated with 16-weeks treatment with peginterferon and flat-dose ribavirin without compromising the chances for SVR. Additional trial is required to optimize the duration of treatment.

PP-471

Study of the correlation between nitric oxide profile degree of liver injury and structural vascular changes of the gastric mucosa in chronic hepatitis c virus related liver disease

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Background: The mechanisms underlying liver injury and fibrogenesis in chronic hepatitis C virus (HCV) infection are still poorly understood.

Aim of the work: This study was designed to correlated serum nitrite and nitrate levels with the degree of liver injury and gastric mucosal changes in HCV patients.

Subjects and Methods: 80 HCV infected patients were classified equally into 4 groups; chronic hepatitis C, Child A,B and C cirrhotic groups. 20 healthy subjects were allocated as a control group. For all patients, serum nitrite and nitrate levels, HCV RNA and liver test profile were evaluated and for chronic hepatitis and Child A cirrhotic patients, liver biopsies were obtained for grading, staging and expression of INF-γ and pentosidine. Gastric mucosal biopsies to evaluated for the degree of (PHG) and expression of vascular endothelial growth (VEGF).

Results: Serum NO profile was significantly higher in all HCV infected patients than healthy subjects and was correlating with the severity of Child-Paugh classification. . Also, hepatic pentosidine expression was correlating with staging and fibrosis. . Also both of serum NO and gastric VEGF were over expressed and correlating with the degree of PHG.

Conclusion: In HCV infected patients, serum NO was significantly correlating with the severity of chronic liver disease. Pentosidine might be considered a marker of oxidative stress.

PP-472

Involvement of alpha-actinin in hepatitis C virus replication is dependent on its non-phosphorylation status

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Our previous studies have reported that host protein alpha-actinin is involved in HCV replication. However, its molecular mechanisms are still unknown. In this study, we first confirmed the role of alpha-actinin in HCV RNA replication with HCVcc system. By transfection of small interfering RNA against alpha-actinin, we found that the amounts of HCV RNA and proteins (NS5A and NS3) were markedly reduced in both HCV replicon and HCVcc cells. Knock-down of alpha-actinin in Huh7.5 cells significantly reduced HCV virus replication and secretion. As alpha-actinin is phosphorylated on the tyrosine residue and phosphorylation can reduce the binding of alpha-actinin to actin, we further investigated if the phosphorylated alpha-actinin was different between HCV replicon and Huh7 cells and whether it influenced on HCV RNA replication. Co-IP results showed that the phosphorylation level of alpha-actinin in replicon cells is much lower than that in Huh7 cells. Over-expression of alpha-actinin dephosporylation mutant (Y12F) enhanced the expression of NS5A and NS3. Membrane flotation assay shown that the Y12F mutants were cofractionated with HCV NS proteins on lipid raft fraction. These results indicated that HCV

replication may influence phosphorylation status of alpha-actinin and its association with actin to regulate HCV replication.

PP-473

Efficient induction of mouse immune responses to hepatitis C virus by viral core protein-carrying attenuated S. typhimurium

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HCV core protein is considered to be an attractive candidate for inclusion in a protective vaccine. In this study, a eukaryotic expression plasmid pCI-C, and an in vivo-inducible prokaryotic expression plasmid pZW-C for HCV core protein were constructed and transformed into an attenuated *Salmonella typhimurium aroA* strain SL7207. The recombinant bacteria, SL7207/pCI-C and SL7207/pZW-C, were used to orally immunize BALB/c mice, and immune responses were assessed. Immunization with bacteria SL7207/pCI-C led to persistent decrease of $CD3^+CD4^+$ T cells and triggered weak anti-core IgG production. Splenocytes from SL7207/pCI-C immunized mice developed relatively weak proliferation response and inferior cytotoxic activity compared with those from SL7207/pZW-C immunized mice. The results suggest that de novo host synthesis of native HCV core protein may cut down the induction of immune responses. Attenuated *S. typhimurium* carrying HCV core protein could efficiently activate systemic cellular and humoral responses, and may be a promising strategy for the development of core-based HCV vaccines.

PP-474

Effects of systematic nurse-provided therapeutic education on adherence and efficiency of PEG-Interferon-a2a (Pegasys®)-ribavirin treatment in chronic hepatitis C (Pegobs Protocol)

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Introduction: Failures of PEGIFN-α2a/b-ribavirin combination in chronic hepatitis C are mainly due to a poor adherence and side effects. No prospective study has still been performed for assessing the effect of systematised therapeutic education.

Aim: to assess the effect of systematic nurse-provided therapeutic education on adherence and efficiency of PEGIFN-α2a (Pegasys®)-ribavirin in patients with chronic hepatitis C.

Patients and methods: muticentric, prospective, randomized study : GrA: systematic nurse consultation after medical consultation at D0, W4, 8, 12, 24, 36; performed with a standardised questionnaire for the assessment and education on the disease and its TT; GrB: pragmatic follow-up.

Results: 239 patients GrA 123 ; GrB 116.

Adherence to TT better ($p<0,05$) (GrA vs GrB) at W24 : 75,6vs64,5%, end of treatment (EOT) 73,7vs61,9%. Accordance of TT duration with protocol for 24 W : 83,6vs76,7% ; 48 W : 69,7vs53,2%. HCV RNA disapparence was more frequent ($p<0,01$) in GrA vs GrB at W12 : 72,8vs57,1% ; W24 : 75,2vs59,8% ; EOT : 70,6vs52%.

Sustained virological response (SVR) was significantly higher: 37,7vs25% for all pts; naïve : 46,4vs31% ; re-TT : 25vs16%. SVR according to genotypes: HCV1, 4,5 : 30,7vs14% ; HCV 2, 3 : 50vs43%. No difference according to the stage of fibrosis.

Conclusion: the systematic nurse-provided therapeutic education is significantly associated with a better adherence to the TT and a better virological response. The beneficial effect was more significant in patients treated 48 W.

We thank Roche Laboratory for its support.

PP-475

Alternate reading frame protein (ARFP) expression in HCV Genotype 3 infected patients

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HCV is a life threatening disease with approximately 170 million people affected worldwide. In Pakistan the prevalence of HCV has been reported to be approximately 6.5%, with genotype 3 being the predominant strain. During HCV infection a +1 frame shift in the Core reading frame leads to the synthesis of a new protein called Alternate Reading Frame Protein (ARFP). Even though ARFP is known to be expressed yet its function remains unknown. Using samples from HCV genotype 3 patients as a source, we cloned and expressed ARFP in bacteria. This protein was then subsequently used as an antigen to detect anti-ARFP antibodies in patients sera which signifies its potential role in diagnostics or as a vaccine component. Currently, work on ARFP expressing transfected cell lines is in progress that will help in identifying its contribution in HCV replication and persistence. The outcome of this study would open new avenues for therapeutic intervention against HCV disease progression.

PP-476

Interaction of non-nucleoside inhibitors with HCV polymerase NS5B: Effect of resistance mutations on compound binding affinity and identification of slow binding inhibitors

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Antiviral potency of non-nucleoside inhibitors (NNIs) of HCV polymerase NS5B is likely related to NNI-NS5B binding affinity. To systematically assess the relationships between NNI binding and antiviral potency, four classes of NNIs representing the palm- and thumb-binding sites were determined for binding affinities to NS5B variants. A robust NNI-NS5B binding assay was developed based on quenching of NS5B intrinsic fluorescence. Compound potencies were determined as inhibition of *in vitro* RNA synthesis activity and HCV replicon replication. The results showed that NNI binding affinities to NS5B correlated well with their inhibitory potencies on HCV polymerase and replicon replication. HCV-796 was unique, showing slow binding kinetics with 27-fold increase on NS5B binding affinty in 3hrs. The inhibition potency on the polymerase activity increased accordingly by preincubation of NS5B and the compound. The signature mutations on the NS5B backbone specifically reduced the binding affinities of NNIs at their respective binding sites. In summary, reduction of NNI-NS5B binding affinity is a key mechanism to achieve NNI resistance. HCV-796 is differentiated from the other NNIs by converting to a potent, high affinity inhibitor through slow-binding of NS5B.

Figure: Effects of HCV NS5B resistance mutations on the binding affinities of HCV-796 as compared to the inhibition of NS5B polymerase activity and HCV replicon replication. (A) K_d fold changes of HCV-796 binding to resistance mutant proteins as compared to wild-type NS5B-Con1 and the IC_{50} fold changes on NS5B polymerization and HCV replicon replication. White bars: K_d-fold changes; net bars: IC_{50}-fold changes on NS5B polymerase activity; black bars: IC_{50}-fold changes on HCV replicon. (B) The locations of resistance mutations in the structure of NS5B.

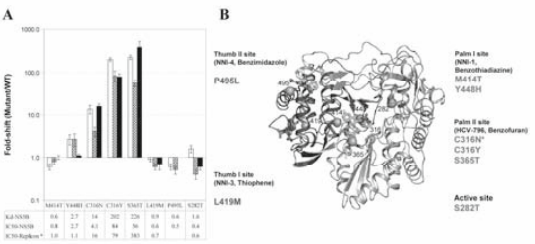

Table. The binding affinities of structurally differentiated HCV compounds are correlated with the inhibitory potencies on the RNA polymerase activity of NS5B570-Con1

Compound structural class	Binding site	Structure	NS5B570-Con1 (FQ) K_d [μM][a]	NS5B570-Con1 (Pol) IC_{50} [μM][a]
NNI-1 Benzothiadiazine	Palm 1		0.019 ± 0.01	0.009 ± 0.03
HC-796 Benzofuran	Palm 2		0.071 ± 0.028[b]	0.081 ± 0.019
NNI- 3 Thiophene	Thumb 1		0.026 ± 0.011	0.17 ± 0.028
NNI- 4 Benzimidazole	Thumb 2		14 ± 2	28 ± 4

[a] Mean values and standard deviations for kinetic constants determined from at least three experiments.
[b] The K_d value of HCV-796 was 1.9 ± 0.2 μM at 10 min of incubation with NS5B-Con1, and it reduced to 0.071 ± 0.028 μM at the binding equilibrium of 3 hr incubation. No time dependent changes in binding affinity were observed for other classes of NNIs.

PP-477

Total and LDL cholesterol are independent predictors of rapid virological response to standard therapy with peginterferon and ribavirin in HCV patients

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Rapid virological response (RVR) is becoming a new tool for predicting treatment outcomes in HCV patients. Aim of this study was to evaluate predictive factors of RVR in HCV difficult-to-treat (genotypes 1/4) and easy-to-treat (genotypes 2/3) patients.

Fifty-one patients (27 genotype 1/4, and 24 genotype 2/3) were treated with pegIFN-alpha2a or 2b once weekly plus daily ribavirin based on body weight for 24 or 48 weeks, according to genotype. RVR was defined as undetectable HCV-RNA at week 4.

Twenty-four patients achieved RVR (37.0% in genotypes 1/4 and 58.3% in genotypes 2/3, p<0.02). Rapid viral responders were younger and had higher mean serum total-cholesterol and LDL-cholesterol levels, as compared to non-responders (p<0.01 and <0.002, respectively). Before treatment, total-cholesterol was 150.7±32.7mg/dl in patients without RVR (12 pts), 166.8±22.6mg/dl in HCV-RNA positive patients showing a ≥2 log decrease of viral load at week 4 (15 pts) and 195.4±46.6 mg/dl in those with RVR (24 pts) (p<0.005). Prevalence of RVR was 29.4% in the bottom tertile of total-cholesterol and 81.3% in the top tertile (p<0.005). No significant associations were found between RVR and BMI, waist circumference, diabetes, cirrhosis, blood pressure, HOMA-IR, ALT and viral load and type of PegIFN-alpha administered. Only age, LDL-cholesterol and HCV genotype were independently associated with RVR.

PP-478

Central underfilling in preascitic HCV cirrhosis with clinically significant portal hypertension: Implications for a cautious approach to antiviral management

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The systemic/portal hemodynamic profile of Child A postviral cirrhosis with diastolic dysfunction (reduced E/A ratio<1.0 at Doppler echocardiography) is largely unknown.

Aim: to assess hepatic/systemic hemodynamics in post-viral compensated cirrhotics with diastolic dysfunction candidated to PegInterferon/Ribavirin facing the impact of Ribavirin-induced anemia on systemic hemodynamics.

Patients and methods: 20 males (53.7±2 yrs) with HCVRNA+ Child A cirrhosis, reduced E/A ratio (0.9±0.05) and F1 varices were studied. Hepatic venous pressure gradient (HVPG), cardiac output (CO), cardiac index (CI), systemic vascular resistances (SVR), pulmonary arterial pressure (PAP), pulmonary arterial wedge pressure (PAWP), right atrial pressure (RAP), pulmonary vascular resistances (PVR), mean arterial pressure (MAP), heart rate (HR) were measured.

Results: HVPG was 14.7±2mmHg. Systemic hemodynamics were normal except pulmonary vascular resistances: CO: 6.6±0.2 l/min (3-7), CI: 3.1±0.1 L/min/m^2 (2.4-4.5), SVR: 1203±46 dyne/sec/cm^{-5} (1100-1500), PAP:11.7±0.6 mmHg (10-18), PAWP: 6.1±0.5 mmHg (2-12), RAP: 2.6±0.5 mmHg (0-5), PVR: 81.95±7.0 dyne/sec/cm^{-5} (120-250), MAP:90.2±3.1 mmHg, HR:72.5±3.6 b/min (60-100 b/min). HVPG correlates inversely with PAP (r=-0.7; p<0.01), PAWP (r=-0.6; p<0.01) and with RAP (r=-0.4; p<0.04). Patients were subgrouped according to a cut-off value of HVPG of 10 mmHg: group 1, mean HVPG 6.3±0.1; group 2, mean HVPG 15.3±0.6 (p<0.001). Patients with higher HVPG showed lower PAWP (5±0.6 vs 7.8±0.7, p<0.01), PAP (10±0.7 vs 14.3±0.6, p<0.01) and RAP (1.7±0.7 vs 4±0.7, p=0.05).

Conclusion: underfilling of central circulation characterizes patients with high HVPG and diastolic dysfunction. Ribavirin-induced anemia may hamper hemodynamic balance via development of hyperdynamic circulation offsetting the clinical benefits of viral clearance in cirrhotics with high HVPG.

PP-479

Integrated backscatter (IBS) tissue analysis in HCV preascitic cirrhosis: Evidence of cardiac hypertrophy

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Diastolic dysfunction is a key feature of cirrhotic cardiomyopathy. Nevertheless the nature of the anatomic abnormality has not been elucidated. In 109 patients with chronic HCV infection E/A ratio, a Doppler marker of diastolic dysfunction, was decreased in cirrhotics (0.89 ± 0.03 vs controls 1.21 ± 0.07, p < 0.01) and to a lesser extent in patients with advanced liver fibrosis (1.17 ± 0.07, p < 0.01). Left ventricular parietal wall thickness was increased (1.80 ± 0.4 vs 2.03 ± 0.3 cm, p < 0.01). While cardiac hypertrophy has been described in the BLD rat model, the pattern of heart tissue abnormality in human cirrhosis has never been investigated in *in vivo* studies. To this aim we employed the echocardiographic IBS technique to obtain an indirect estimate of tissue density (decreased when a higher percentage of muscle fibres is present and increased when fibrosis prevails) to provide myocardial tissue characterization in a subset of 31 pts with compensated HCV cirrhosis. The portal venous pressure gradient was measured in those patients with indirect signs of portal hypertension (mean HVPG 15 mmHg). The average IBS signal was overall reduced in cirrhotics at the level of the posterior wall (21.72 ± 1.46 dB versus 30.85 ± 1.40 dB in controls, p < 0.01), irrespective of high HVPG . Our results confirm diastolic dysfunction in HCV cirrhosis pointing to cardiac hypertrophy as the anatomomic background, at least in the compensated stage of disease.

PP-480

The study on detecting Hepatitis E virus related genotype and subgenotype among animals in Guangxi

Xianfei Wei

Objective: To detaction of HEV among swine, rats, dogs, macaque, fishes and the related genotype and subgenotype in Guangxi.

Methods: HEV RNA was amplified by a reverse transcription nested polymerase chain reation (RT-nPCR), and RT-nPCR products were cloned and sequenced. The nucleotide sequence comparison and phylogenetic analysis of HEV isolated from animals were carried out by using the Vector NTI Suite 9.0 and TreeView softwares.

Results: The HEV RT-nPCR of all 170 serum samples of swine, rats and dogs were detected negative, and HEV RNA was also negative among all the remaining samples, including, 140 liver tissue samples of rats, 120 bile samples of fishes, 80 dogs and 130 fecal samples of macaque. The HEV positive rate of fecal samples in swine was 10.08% (13/129). These 13 swine HEV ORF1 nucleotide sequences islates shared similarities of 73～77%，76%～79%，76%～81%，82%～97% with genotype I-IV, respectively. The similarity with 6 subgenotypes were 4a:82～94%，4b:82～98%，4c:82%～87%，4d:81%～87%，4f:84-86%，4g:81%～84%, respectively. Among those 13 swine isolates, 2 isolates shared the highest similarity as 82~94% with IVa and 11 isolates shared the highest similarity as 82~98% with IVb.

Conclusion: HEV strains of swine from Guangxi were classified as HEV genotype IV, 2 of which were classified as HEV IVa subgenotype and 11 of which were classified as HEV IVb subgenotype.

PP-481

Establishment of a real-time monitoring system for kinetic characterization of RNA-cleaving DNAzyme

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Background: In the present study, we reported the establishment of a real-time monitor system for directly observing the catalytic, kinetic characteristics of DNAzyme 10-23 in vitro cleavage on the target RNA molecules as well as for rapid, accurate, high-throughout evaluation of varied DNAzymes on their counterpart RNA molecules.

Methods: DNAzyme named Dz-HCV-9 specific to hepatitis C virus (HCV) ORF AUG were designed and synthesized. Dz-HCV-Mis-9 with mismatched substrate-recognition domains, Dz-HCV-Mut-9 with mutant catalytic domains, antisense oligonucleotide ASON and nonsense oligonucleotide NSON were synthesized respectively as controls. A chimeric oligonucleotide of 29nt containing both RNA and DNA bases was designed and synthesized as the substrate: 5' FAM-GT AGACCGUGCACCAUGAGCACGAAUCCT-BHQ 3', corresponding to the330—354 nt (underline) of HCV genome(gi: 329873) The reporter FAM/BHQ was incorporated at the 5' and 3' end, respectively. Under simulated physiological conditions (37℃), kinetic characterization of RNA-cleaving DNAzyme was analyzed in a real-time way. Factors that influencing DNAzyme cleavage were analyzed.

Results: Dz-HCV-9 specific to HCV ORF AUG could cleave target RNA at A•U site, a continuous change of fluorescence intensity was monitored. While the control oligonucleotides couldn't cleave RNA, there were no change of fluorescence intensity. Factors that influencing DNAzyme cleavage concluded different substrate-recognition domain, Mg^{2+}concentration and pH.

Conclusion: A real-time monitoring system for kinetic characterization of RNA-cleaving DNAzyme was successfully established in the first time.

PP-482

IL-18 levels and their clinical significance in pegylated interferon alpha 2a-treated patients with chronic viral hepatitis C

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Aim: The aim of this work was to analyze the serum levels of proapoptotic interleukin (IL)-18 in patients with chronic hepatitis C (CHC), at baseline and after treatment with pegylated interferon alpha-2α (PEG-IFN).

Methods: Thirty-one patients with CHC were studied, at baseline and after treatment with pegylated interferon alpha-2a (PEG-IFN) for 24 weeks. A group of 15 healthy sex- and age-matched individuals was selected as control. Evolution of the serum concentrations of IL-18 was analyzed after treatment. Response to treatment was assessed according to clinical and virological changes.

Results: Fifteen (48.3 %) achieved sustained response by 24 weeks PEG-IFN treatment, while 8 (25.8 %)patients were relapsers and 8 patients were non-responders (25.8 %).All patients with CHC had raised IL-18 levels comparing controls（113.4±30.4 pg/ml versus 66.9±19.5 pg/ml; $P<0.01$）. Serum IL-18 levels showed a positive correlation with serum ALT levels($r=0.66$, $P<0.05$). In sustained responding group, a significant decrease of IL-18 serum levels was observed at week 12 and 24. Increased levels persist in those in whom the HCV infection was not eliminated by the therapy.

Conclusion: The trend of the serum levels of IL-18 show the severity of liver inflammation, but also can be used as the index of investigating the therapeutic effect of PEG-IFN treatment. The patients whose serum level of IL-18 is higher before treatment are more possible to get lasting-respondence.

PP-483

Interferon γ inhibits Hepatitis C virus infection by down-regulating claudin-1

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Hepatitis C is a major cause of chronic hepatitis worldwide and current medical treatment options are limited. Claudin-1 (CLDN1), a tight junction (TJ) protein that is involved in the formation of TJ barrier function and highly expressed in the liver, was recently identified as a co-receptor for HCV entry. Proinflammatory cytokine interferon γ (IFN-γ) has been implicated in TJs disruption. Since CLDN1 is proposed to be involved in barrier maintenance, polarized Caco-2 cells, demonstrated to support HCV entry, were utilized to study the influence of IFN-γ on TJs and cell culture-derived HCV(HCVcc) infectivity. Epithelial barrier disruption was induced by IFN-γ in a time-dependent manner as demonstrated by measurement of transepithelial electrical resistance and dextran permeability. Confocal microsocpy showed that IFN-γ treatment led to significant redistribution of CLDN1, CD81 and SR-B1. In addition, western blot analysis revealed that the expression of CLDN1 was decreased. IFN-γ treatment reduced the susceptibility of Caco-2 cells to HCVcc infection. These results indicate that IFN-γ may be crucial in the inhibition of HCV infection by regulating the expression of reduction regulating CLDN1 expression and HCV receptors distribution.

PP-484

Insulin resistance in hepatitis C: fibrosis progression and response to treatment

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Background: Evidences support a relationship between insulin resistance (IR) and hepatitis C. Insulin resistance was correlated with fibrosis progression and a non response to anti-viral treatment.

Aims: to assess the association of insulin resistance (IR) with fibrosis development and with sustained virological response to treatment with Interferon alfa plus Ribavirine.

Methods: We analyzed 58 hepatitis C infected patients with hepatic biopsies: group 1 with Body Mass Index (BMI) ≤ 30 with study of Homeostasis Model Assessment Insulin Resistance (HOMA-IR) post treatment. Group 2: 33 with BMI > 30, 15 with and 18 without diabetes assessed HOMA-IR 4 times pre, per and post-treatment. We assessed relationship between IR and fibrosis stage by METAVIR score and between IR and therapeutic response. Statistical analyze: X square, Student test and Mann Witney. **Results:** group 1 presented association between IR and BMI> 29.27kg/m2, waist-to-hip ratio >97.72cm, fasting insulin >18.69μUI. At the group 2, advanced fibrosis (F3 F4) was associated with aspartate aminotransferase (AST) > 96.84IU and genotype 1. When compared advanced fibrosis between the group 1 and 2, fasting blood glucose >100mg/dl, waist-to-hip ratio >105 cm, BMI>32. kg/m and AST >109,0UI were associated with F3 F4. A worse response to treatment was associated with: HOMA-IR >3.10, hepatic steatosis (89,5%), and genotype 1 (94,7%).

Conclusion: Advanced fibrosis was associated with obesity and high fasting blood glucose level. Genotype 1 carriers had higher HOMA-IR and it was associated with advanced fibrosis. HOMA-IR>3.10, high anthropometrics indexes, steatosis and genotype 1 were associated with worse response to therapy.

PP-485

The bile duct lesions in chronic viral C hepatitis

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Introduction: In Romania, the etiology of chronic hepatitis is dominated by the hepatitis C virus, sometimes associated to the hepatitis B virus.

General epidemiological research place our country in an area of intermediate prevalence for the HCV infection (4,9% in the general population) and around 1 million people are infected with HCV.

Aim: to identify and semiquantitative grading of the bile duct lesions and evaluating their relationships with the demographical, biochemical, virusological and morphological characteristics.

Patients and methods: We studied 242 naïve patients admitted in our hospital in 2003-2006 for chronic hepatitis C, who performed biochemical and virusological profile and liver biopsy for diagnosis, being evaluated according the Ishak system.

Results: The involvement of the bile ducts was identified in 69.8% of the total patients, especially as contour lesions (42.1%) and less as necrosis (11.2%) or proliferation (16.5%).

The bile duct lesions were significantly associated with the patients' age and the AST level ($p = 0.006$). No significant associations with the mean ALT, GGT or viremia levels were observed.

The involvement of the bile ducts associated to a significant extent to the necroinflamation, portal inflammation and fibrosis levels, as well

with the presence of lymphoid aggregates. No association with steatosis was found.

Conclusions:

1. The bile duct involvement was identified in 69.8% of the patients.
2. As far as the lesions severity is concerned, the most frequent were the contour alterations (42.1%) and less frequent the necrosis (11.%) or proliferation (16.5%).
3. The bile duct lesions was significantly correlated to the age, the increased AST, the necroinflamatory activity, portal inflammation, fibrosis and the presence of lymphoid aggregates.

PP-486

Impact on RNAi by the adaptive evolution of hepatitis C virus (HCV)

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Immune-escaped mutations have been revealed in Hepatitis C Virus (HCV). In order to explore how HCV escaping from host immune surveillance by rapid variation, we analyze the protein-coding sequences of HCV from 1a and 1b subtype worldwide, and identify 36 amino acid sites under positive selection by modified Suzuki-Gojobori method. According to the epitope maps, sites under positive selection are mapped onto immune epitope. The result suggests adaptive mutation evolution of HCV is the consequence of the environment pressure directly driven by host immune response. The 14th site of E2 protein is detected both in 1a and 1b subtypes. Since this site and the 8th site of E2 protein have been reported in our previous study, they could be the key mutation sites which correspond to HCV escaping from immune surveillance. So far, the effective therapy against HCV is using virus mRNA translation inhibitor, IFN-α, and anti-sense RNA also reported to applied to specific therapy. Therefore, future therapy would be suppression the HCV protein expression and proliferation by RNAi strategy. However, high mutation rates of HCV will cause the target-off effect. On the other hand, new target seed sequences may be appeared through mutation. In this work, we simulate the point mutations on adaptive sites, and predict the potential patterns for target-on and target-off effect. After statistical comparison, we evaluate the impact on RNAi by the key mutations of HCV. The newly arisen target-on regions could be the candidate vaccination targets.

PP-487

Toll-like receptor-dependent IFN I and III subtype induction in human peripheral blood mononuclear cells from patients with HCV infection

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Toll-like receptors (TLR3, 7, 8, 9) have a critical role in innate immunity against virus. TLR3 recognizes viral double-strand RNA. TLR7/8 recognizes viral single-strand RNA. TLR9 recognizes viral double-strand DNA. All these TLRs induce type I and III interferon (IFN) for antiviral reaction. For chronic hepatitis C virus (HCV) infection, IFN-a combined with ribavirin is the standard treatment regimen. We investigated the 21 type I and III interferon (IFN) subtypes in patients with HCV infection, with or without sustained response to IFN-a treatment. Peripheral Blood Mononuclear Cell (PBMC) were obtained from 20 HCV patients with sustained response (SVR) , 12 non response (NR) or relapse, and 22 healthy controls and stimulated by TLR3-ligand (Poly I:C) , TLR7/8-ligand (CL097) , TLR9-ligand (CPG-DNA). Total RNA was extracted from the PBMC, reversed transcript to cDNA. Quantitative PCR assays were done to detect the cDNA. IFN Beta expressed higher in SVR than others after 2 hour's induction by Poly I:C (p=0.0243), , IFN a2 expressed higher in SVR by Poly I:C 6 hours (p=0.0418), lambda1 expressed higher in SVR than in control by CL097 2 hours (p=0.0278). Lambda1 and a17 expressed lower in control than others. Upon different Toll-like receptor activations, IFN a2(TLR3), a17(TLR7/8), Beta(TLR3), lambda1(TLR7/8) were higher induced in SVR HCV patients, compared with healthy controls or NR/relapse, indicating the different roles of these IFN subtypes in human anti-HCV immunity or the response to IFN treatment.

PP-488

Absence of early virological response is independently predict non-response to treatment in genotype 4-infected chronic hepatitis C patients

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AIM: To evaluate the impact of early virological response (EVR) in the sustained virological response (SVR) rates of treatment-naïve, chronic hepatitis C (CHC) patients infected by genotype 4, treated with pegylated interferon-a2b (PEG) plus ribavirin (RIB), under real life conditions in Greece.

METHODS: 72 consecutive CHC patients (75% males, 9.3% cirrhotics, 73.6% with HCV-RNA>600000 IU/ml) who had been treated with PEG plus RIB, for 48 weeks, were retrospectively analyzed. EVR was confirmed by undetectable serum HCV-RNA or at least 2 log drop of HCV-RNA, compared to baseline values, at week 12 of treatment. Statistical analysis was based on t-test, chi-square analyses and logistic regression analyses ($p<0.05$).

RESULTS: 80.6% of patients complete the treatment schedule. 53.4% were characterized as responders whereas 29.3% were characterized as non-responders and 17.2% as relapsers. Patients who exhibited EVR were younger ($p=0.035$), had lower baseline viral load ($p<0.0001$) and less fibrosis score ($p=0.04$) in liver biopsy than those with non-EVR. In the univariate analysis non-response were significantly associated with older age ($p=0.038$), lower histological activity index ($p=0.038$), presence of cirrhosis ($p=0.008$) and lower EVR rates ($p<0.0001$). In the multivariate model that was adjusted for all the baseline parameters only the absence of EVR ($p=0.006$, 95% CI, 0.001-0.305) independently predicted non-response.

CONCLUSION: Absence of EVR independently predicted non-response in treatment-naïve, genotype 4-infected CHC patients, treated under "real life" conditions with the currently approved combination treatment, irrespective of epidemiological, virological and histological baseline data.

PP-489

Differential expression of the CXCR3 ligands in chronic hepatitis C, and their modulation by HCV replication in vitro

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The factors that regulate lymphocyte traffic in the HCV infected liver are not completely defined, however the CXCR3 ligands, I-TAC, IP-10 and Mig are believed to play a major role in T-cell recruitment into the liver. The AIM of this study was to investigate the relative expression of the CXCR3 ligands in chronic hepatitis C (CHC) and investigate the effect of HCV on their expression in vitro.

Results: Microarray and real-time PCR analysis of the HCV infected liver demonstrated that mRNA for all three CXCR3 ligands was significantly increased compared to normal human liver. Expression correlated with lobular inflammation, however, only I-TAC expression correlated with portal inflammation. CHC serum analysis revealed that I-TAC and Mig levels were not significantly elevated in the periphery, which was not the case for IP-10, demonstrating that I-TAC and Mig may form more of a chemokine gradient between the periphery and liver. Luciferase reporter assays and real-time PCR demonstrated that replicating HCV in the presence of IFN-gamma/TNF stimulation was able to modulate I-TAC expression, but not that of IP-10 and Mig. This selective increase in ITAC expression was mediated by the cellular dsRNA response.

Conclusions: The CXCR3 chemokines are highly expressed in CHC and are likely to be the riving force in T-cell recruitment into the liver. Furthermore HCV replication (in vitro) is able to modulate CXCR3 chemokine expression in a positive manner, and may play a role in the upregulation of these molecules seen in CHC together with IFN-gamma and TNF.

PP-490

Predisposition of HLA alleles to HCV genotype in Korean patients with chronic HCV Infection

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Background/Aim: Human leukocyte antigens (HLAs) as well as hepatitis C virus (HCV) genotype have been reported to influence the clinical outcome of HCV infection. The aim of this study was to investigate whether particular human leukocytes antigen (HLA) molecules are associated with predisposition to HCV genotype in Korean population.
Methods: One hundred-forty patients with chronic HCV infection and 208 normal individuals were examined for HLA class I and –II molecules.
Results: Of total patients with chronic HCV infections, the number of genotype 1b and -2a were 85 and 55, respectively. In class I antigens, the frequencies of HLA-B46 significantly increased in patients with genotype 2a compared to those with genotype 1b (16.4 vs 4.7 %, $P < 0.05$). The frequencies of HLA DRB1*0406, -DQA1*0103 and –DQA1*0301 were significantly higher in patients with genotype 2a than those with genotype 1b (12.7 vs 2.4%; 34.5 vs 16.5%; 25.5 vs 10.6%, respectively, $P < 0.05$). On the other hand, the frequencies of DPB1*0604 was significantly lower in patients with genotype 2a compared to genotype 1b patients (5.5 vs 18.8%, $P < 0.05$).
Conclusions: Our results demonstrated that particular HLA alleles are associated with predisposition to HCV genotype. These findings provide new insights to understanding of underlying immunopathogenesis of HCV infection.

PP-491
Clinical analysis of elderly patients with chronic hepatitis C
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Objective: To evaluate clinical features and treatment Of chronic hepatitis C (CHC) in the aged patients
Methods: Clinica1 data of 94 patients diagnosed with CHC was reviewed. Elderly patients(≥60 years)were compared in terms of clinical，biochemical，immunologica1 features and treatment with younger patients(< 6Oyears)
Results: Among the 94 patients, the initial diagnosis of CHC was made in 51 patients at an age≥60 · The elder patients had similar clinica1 manifestations，except for higher liver cirrhosis ratio compared with younger patients(39.2％ VS 16 · 3％，*P*< 0 · 01) · Diabetes and Hypertension occurrence are higher in elder patients than in younger patients. The serum leve1 of white blood cell and platelet count was lower in elderly patients compared with that of younger patients[(3.6±1.9) $\times 10^9$/L VS(5.8±1.3) $\times 10^9$/L and （140±80）$\times 10^9$/L VS（220±60）$\times 10^9$/L，respectively，P<0 · 01]，whereas there were no statistica1 differences in the serum HCV RNA levels, serum alanine aminotransferase, the serum total bilirubin and immunological findings between older and younger patients (*P*>O · 05) · The ratio accommodate Peg-interferon alfa-2b plus ribavirin therapy lower in the elderly than in younger group(35.3％ VS 81.4％，P< O · O1) · among therapy Discontinuation or dose reduction was more frequent in older patients (*P*<O · 05).
Conclusions: Elderly patients have more complication compared with younger patients ·They have lower tolerance with combination antiviral therapy compared with younger patients . This suggests that we should diagnose and treatment CHC early.

PP-492
High incidence of typhoid in hepatitis C patients living in poor hygienic
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Origin of Hepatitis C is unknown in more than one third cases. Pollution is suspected as a cause and the objective of this study find a significant indirect evidence that could relate it with spread of HCV. We thought that typhoid being a proven pollution related disease, determination of its coinfection in HCV patients living in a community with poor hygienic conditions would be of interest. The typhoid parameters included were PCR, blood culture and Widal test. In the primary study there were three groups from a locality with poor hygienic conditions, PCR and ELISA positive patients of HCV (225) – further subdivided into two groups, with history of known causes of transmission (150), and without any such history (75); clinically diagnosed cases of typhoid (75); and healthy controls (100). In a secondary study, another group of 200 healthy individuals from the same locality and 200 healthy individuals from a locality with good hygienic conditions was included to compare exposure to both diseases by antibody screening methods. The increase in these parameters for HCV cases as compared with normal subjects was statistically very significant as was the difference between two groups of HCV patients. The results for the secondary study were also statistically significant. These results indicate that the source of infection for the two diseases is same in many cases in areas with poor hygienic conditions and exposure to one disease does not make the subject susceptible to other. Therefore, a relationship between pollution and Hepatitis C is strongly suggested.

PP-493
Discovery of the missed link between schistosomiasis and hcv infection
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Schistosomiasis is a term covering infestation of man by blood fluke trematode parasite.
Egypt S. mansoni occurs intensively in the Nile Delta, while S. hematobium is endemic in Nile Valley.
Studies in Egypt found the highest risk of HCV infection in whom infested with schistosomiasis. HCV-Ab prevalence reported 70% in adults suffering from Schistosomiasis and without history of blood transfusion.
Determine the link between schistosomal infestation and HCV, whether the parasite could be vector of transmission of the virus to human
Different stages of life cycle of S. mansoni provided by Theodore Bilharzias Institute.
Specimens of living S. mansoni worms and Biomphalaria alexandria snails were grounded separately in sterile mortar after adding 5 ml. Sterile saline. After centrifugation sterile supernatant tested for
Detection of HCV-RNA by RT-PCR
HCV-RNA quantitation
HCV-Antigen: Worms , Miracidia , Snails and Cercariae of S. mansoni were Positive for HCV-Ag.The Snails gave strong positive result.Eggs gave negative result.
HCV-RNA by RT-PCR: Worms , Miracidia , Snails and Cercariae of S. mansoni tested for HCV-RNA By Qualitative RT-PCR were Positive. The eggs gave negative result.
HCV-RNA Quantitation: Miracidia were positive (800 Copies/ml) and Snails were positive (1100 Copies/ml) other specimens gave negative results
Existence of virus and its replication in parasite
S. mansoni parasite carries HCV and considered as a non-human vector for transmission of HCV infection
Parasitic and viral co-infection change pathology of hepatic schistosomiasis from periportal fibrosis to cirrhosis and the development of HCC.

PP-494
Phase 2 studies with albinterferon Alfa-2b (alb-IFN) dosed q4wk provide insights into dose selection for future Studies
Jenny Heathcote[1]
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Phase 2 studies of alb-IFN, a novel, long-acting IFN, were conducted in IFN-naïve CHC patients to assess efficacy/safety of q2wk and q4wk dosing. Study 1 (Gt1 CHC): alb-IFN 1200µg q4wk (n=116), 900µg q2wk (n=118), and 1200µg q2wk (n=110) vs PEG-IFNα-2a 180µg qwk (n=114), plus ribavirin 1000-1200mg/d in all arms. Small, exploratory study 2 (Gt2/3 CHC): alb-IFN 1500µg q4wk (n=22) vs 1500µg q2wk (n=21), plus ribavirin 800mg/d. Study 1: SVR rates with 1200µg q4wk vs 900µg, 1200µg q2wk, and PEG-IFNα-2a were 51% vs 58%, 55%, and 58%, respectively. Although wk4 rapid virologic response rate was lowest with alb-IFN 1200µg q4wk, those patients had 100% positive predictive value for SVR. Viral breakthrough and relapse rates with 1200µg q4wk were comparable to q2wk and PEG-IFNα-2a. Alb-IFN 1200µg q4wk had the least hematologic toxicity. Study 2: q4wk vs q2wk SVR rate: 77% vs 62%. Wk4/12 early response rates with q4wk:

68%/96%. There were no dose reductions due to AEs with q4wk; overall tolerability was favorable vs q2wk. Alb-IFN 1500μg q4wk had less impact on hematologic parameters. Although overall SVR rate with alb-IFN 1200μg q4wk was impressive in Gt 1 CHC, higher doses may be needed to achieve outcomes comparable to weekly PEG-IFN therapy. Alb-IFN 1500μg q4wk demonstrated promising efficacy in small group of Gt 2/3 CHC patients. Both alb-IFN q4wk regimens had acceptable tolerability, with minimal impact on hematologic variables. These data have helped guide design of a dose-ranging phase 2b study of alb-IFN q4wk in IFN treatment-naïve GT 1 CHC.

PP-495

Research on detecting HCV gene and antibody in serum, urine, saliva and semen

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Moreover,19 cases of spouses and 4 cases of children(under 12) are also measured.The 19 cases of male patients whose HCV RNA in serum,saliva and urine are all positive.In consequence,HCV RNA(+) in spouses' serum reach 42.1%,children's 25%,which implies that HCV can spread by close contact with infectious families.So the sexy spreading is possible.The research needs further observation as the semen lacks.

To prevent hepatitis C,it's really valuable to judge transmission,viral copy and detection of asymptomatic carrier that detecting HCV RNA can directly attain the proof of HCV infection.

PCR can find out HCV RNA in the body fluid as well as blood, which offers a new basis to further discuss HCV infectious way.

Determination of both HCV RNA and anti-HCV can develop the diagnosis rate of HCV infection.

To anti-HCV-IgM,early diagnosis is worthless for acute hepatitis C. But it can be treated as the level of Lesions Action and Viral copy,this meaning will be magnificent for guiding clinical treatment.

The author has used the Reverse Transcription and Polymerase chain reaction to detect the HCV RNA in the serum,saliva,urine and semen according to 60 cases of anti-HCV(+) patients with chronic hepatitis.The investigation is also aimed at 23 spouses and children.To judge the possibilities of Non Blood Transmission,the results are as follows:

Figure1.all types of HCV RNA specimens and positive rates of anti-HCV

Specimen	Sample	HCV RNA		Anti-HCV				Occult blood test	
		Positive sample	%	IgM Positive sample	%	IgG Positive sample	%	Negative sample	%
Serum	60	51	85.0	26*	43.3	41*	68.3		
Saliva	60	31**	51.7	2	3.3	2	3.3	56	93.3
Urine	60	21	35.0	1	1.7	0	0	60	100
Semen	5	0	0	0	0	2	40.0	5	100

*anti-HCV-IgM,IgG 7 cases are positive at the same time **4 cases of occult blood tests have been deleted

Figure2.Results of spouses and children

Serum	Sample	HCV RNA		Anti-HCV-IgM		Anti-HCV-IgG		ALT(U/L)
	(23)	Positive sample	%	Positive sample	%	Positive sample	%	
Spouses	19	8	42.1	4	21.1	2	10.5	27
Children	4	1	25.0	1	25.0	0	0	20

Key:5 cases of chronic hepatitis B patiences' anti-HCV and HCV RNA are all negative and HCV RNA in the serum of their spouses and children are also all negative.

PP-496

Association between interferon-induced depression and sustained virological response in patient with antiviral therapy of chronic hepatitis C

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Background: Neuropsychiatric symptoms are commonly associated with chronic hepatitis C virus infection, its sequelae, and its treatment. In particular, interferon, a primary component of treatment for chronic hepatitis C, has been strongly associated with depressive symptoms. The study objective was to determine the contribution of interferon-induced depression to a predictive model of sustained virological response (SVR) in chronic hepatitis C.

Materials and Methods: 14 therapy-naïve hepatitis C virus (HCV) outpatients received treatment with peginterferon alfa-2a and ribavirin. Neuropsychiatric side effects were monitored prospectively (Depression Scale, DSM-IV criteria for major depression). SVR was defined as a failure to detect HCV by PCR 24 weeks after therapy.

Results: SVR rate was 64.3% (9 of 14 patients). Classification data and the extent of interferon-induced depression were not significantly linked to SVR. Virus genotype ($p<0.05$) and gender ($p<0.02$) contributed significantly to a logistic regression model. Mean ($p>0.5$) and maximum ($p>0.5$) depression increases were no significant predictors of SVR. Major depression rates (DSM-IV criteria) were 22.1% (2 of 9 patients) in the subgroup with SVR and 20.0% (1 of 5) in patients without SVR.

Conclusion: We found no significant association between depression and the efficacy of antiviral treatment in chronic hepatitis C. Interferon-induced depressive symptoms are important to be monitored and treated if necessary; however, they cannot be used to predict therapy success.

PP-497

Observation of therapeutic effect of TINMAX HB-3 in patients with hepatofibrosis post hepatitis B and cirrhosis

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Objective: To evaluate the efficiency and safety of "TINMAX" HB-3 Herbal Compound (cpd) in treatment of hepatofibrosis and cirrhosis post chronic hepatitis B.

Methods: A double-blind randomized method was employed .60 patients of hepatofibrosis or cirrhosis post hepatitis B were separated into study group ("TINMAX" HB-3 group) and control group (natural vitamin group) by randomized method. The course was 52 weeks.

Results: 60 patients enrolled in the evaluation.58 patients completed the evaluation according to the protocol. 20 patients had liver biopsies twice, 10 patients in study group and 10 patients in control group. At the end of therapy, the total effective rate of hepatofibrosis in histopathology is 74.13% in study group, much higher than that of 21.95% in control group ($P<0.05$) . The total effective rate of serum markers of hepatofibrosis (HA，LN，PCⅢ，CⅣ)at the end of therapy in study group was 70.10%, much higher than that of 30.24% in control group ($P<0.05$).

Conclusion: "TINMAX"HB-3 herbal compound (cpd) is effective and safe in treatment of hepatofibrosis and cirrhosis post chronic hepatitis B.

PP-498

Regulatory T cells and immune modulation in early hepatitis C infection

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It is not clear what the precise phenotypes of regulatory T (Treg) cells are involved in immune suppression during the early course of HCV infection. Here we show that HCV antigens upregulate *in vitro* an inhibitory Th3 and Tr1 response in early infection. In addition, HCV antigens stimulated higher levels of IL-10 in the subjects chronically infected with HCV. In contrast, IFN-levels was lower in the chronically infected subjects with higher viral loads and higher in the subjects with viral clearance and the subjects infected with lower viral loads. $CD25^+$ Tregs stimulated by HCV antigen expressed higher levels of the transcription factor forkhead box P3 (*Foxp3*) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). Our data indicate that in the early course of HCV infection, a protective peripheral Th1 immune response is related to viral clearance while an inhibitory Th3 and/or Tr1 response is likely critical in viral persistence.

PP-499

Correlation between steatosis, necroinflammatory activity and fibrosis

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Hepatitis C virus is a major causative agent of chronic liver disease. Steatosis is a frequent histologic finding. Its association with fibrosis has been reported, but whether it can be an independent predictor for liver fibrosis is unknown. The aim of the study is to assess the markers of inflammation, fibrosis and steatosis in association with the histopathological changes.

We studied liver biopsies from 350 patients with HCV (age 21-63 years old). The slides were stained using routine and special stainings with antibodies directed against different subsets of T cells, B cells and macrophages, and growth factors. The immunohistochemical markers included UCHL1, CD20, CD4, CD8, CD68 and TGF-beta.

In the piecemeal necrosis the predominant cells were CD4 helper T cells, whereas those seen in the lobule were predominantly CD8 suppressor/cytotoxic T cells. CD20 positive B cells were seen mainly in the portal areas. In the more aggressive forms there was an increased number of CD68 positive cells. The TGF-beta was increased in the foci

of necrosis.
In the group analyses, the association between steatosis and fibrosis invariably was dependent on a simultaneous association between steatosis and hepatic inflammation.
In this group of CHC patients, steatosis is confirmed as significantly and is associated with fibrosis independently of necroinflammation in CHC. Hepatic inflammation may mediate fibrogenesis in patients with liver steatosis.

PP-500
Cross-neutralization antibodies against HCV are present in sera of hepatitis C patients
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A eukaryotic expression plasmid encoding carboxyl terminal-truncated HCV envelope protein 2 (E2) of H77 strain was constructed and transfected into human 293T cells. Both intracellular E2 and secreted E2 protein in supernatant could be detected by western blot analysis with anti-E2 McAb. The supernatant was used to assay anti-E2 antibodies in sera of hepatitis C patients by ELISA and 49 of 60 sera samples was detected anti-E2 IgG positive. The full-length envelope protein expression plasmid was transfected into 293 T cells and the reactivity of transfectant with anti-E2 IgG positive sera were analyzed by immunofluorescence. The intensity of intracellular green fluorescence was paralleled with anti-E2 antibodies level of the tested sera. Four strains of HCV pseudotype particle (HCVpp), including H77 (1a genotype), Con-1 (1b genotype),J6 (2a genotype),and UNK3a (3a genotype), were used to assay the neutralizaion activity of 12 anti-E2 positive sera samples. All the anti-E2 positive sera could neutralize the infectivity of four strains of HCVpp at various degree, and the neutralization activity was consistent with the anti-E2 antibodies levels. The anti-E2 antibodies negative sera did not show obvious neutralization activity. The results suggested that cross-neutralization antibodies against HCV are present in sera of hepatitis C patients, and development of vaccines that induce broadly-reactive neutralization antibodies may be possible.

PP-501
The metabolic profile in chronic viral C hepatitis
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Aim: the evaluation of the prevalence of diabetes and of obesity and identifying the correlations with a biochemical, virusological and morphological profile.
Patients and methods: The 242 patients diagnosed with chronic viral C hepatitis admitted in our hospital in 2003-2006 were questioned, when the history was taken, regarding the associated metabolic pathology (diabetes, dislipidemia and obesity), who performed biochemical and virusological profile and liver biopsy for diagnosis, being evaluated according the Metavir and Ishak scoring systems.
Results: Diabetes type 2 was found in 43 patients (17.77%). Diabetes was associated to the masculine gender (p=0.002) and to the elderly (p=0.00003). About half of the patients had a normal BMI (47.52%), the rest being either overweight (38.84%) or obese (13.64%), without significant differences between the genders.
Regarding the relation between diabetes type 2 and the level of the transaminases, of GGT, of platelets or of viremia there were not significant differences.
Patients with diabetes type 2 have a significantly increased average BMI than patients without diabetes (p=0.006).
Between the presence of type 2 diabetes and the necroinflamatory activity or fibrosis, regardless of the scoring system (Ishak of Metavir), a significant correlation was observed. The diabetic patients present more frequently lesions of steatosis, especially mild or moderate forms (p=0,017).
Conclusions
1. The prevalence of type 2 diabetes in patients with chronic viral C hepatitis was 17.7%.
2. The type 2 diabetes was associated with the masculine gender, the eldery and the high BMI.
3. The presence of type 2 diabetes could not be correlated to the biochemical and virusological profile, but there observed to be significant associations with the necroinflamatory activity as well as with fibrosis and steatosis.

PP-502
Chronic hepatitis C infection in injection drug users in Singapore
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Introduction: Hepatitis C virus (HCV) infection is a potential problem within the injection drug abuse community.
Aims: To evaluate the biochemical, virological and risk factor profile of injection users with HCV infections in Singapore.
Methods: Injection drug users from a rehabilitation programme were voluntarily screened for human immunodeficiency virus, hepatitis B and hepatits C after signing informed consent. Their liver function and biochemical profiles were analyzed.
Results: Of 623 injection drug users, 71.1% were positive for hepatitis C, 1.1% for hepatitis B, 2.5% for hepatitis B and C co-infection, and 0.4% for HIV, hepatitis B and C infections. Of the 433 anti-HCV positive subjects, 34.6% attended our hepatitis C clinic.(92.7% males, 7.3% females;mean age 43 years; 47.3% Chinese, 39.3% Malays, 6.7% Indians).Risk factors: needle use(100%), spouses with hepatitis C(2.7%), sexual promiscuity(58.7%), tattoos(63.3%) and prior blood transfusion(2.7%). 55% shared needles and 79.8% abused multiple drugs.
Virological analysis on 66 subjects showed, 28.7% had undetectable HCV RNA(median RNA 396000IU/ml). 20.9% had genotype 1(2 subtype 1a; 2 subtype 1b; 2 subtypes 1a/1b), 2.3% had genotype 2 (subtypes 2a/2c), 74.4% genotype 3(96% subtype 3a), and 2.3% genotype 4 (subtype 4c/4d). Biochemical analysis was normal (median total bilirubin 10umol/L, albumin 44g/L, AST 42U/L, ALT 65U/L, ALP 77U/L, prothrombin time 13.9 secs, platelet count 266 x10^9/L, AFP 3.8 ug/L). 54 subjects underwent liver ultrasound(39 normal; 10 fatty livers; 4 hepatic cysts; 1 coarse echotexture).
Conclusion: There is a high prevalence of HCV infection in Injection drug users in Singapore. Genotype 3a is the commonest, suggesting transmission of a single strain. Liver function tests are frequently normal and cannot be used to screen injection drug users for active HCV infection.

PP-503
The impact of the duration of ribavirin treatment in the virological response of short term Peg-Interferon treated genotype 2/3 chronic hepatitis C patients
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AIM: To evaluate the impact of the duration of ribavirin (RIB) treatment in sustained virological response (SVR) rates of treatment-naïve, high pretreatment viral load (>800000 IU/ml), genotype 2 or 3 chronic hepatitis C (CHC) patients, who had been treated for 12-16 weeks with weight-adjusted dosing of pegylated interferon (PEG)-a2b (1.5 μg/kg/w) plus flat ribavirin (RIB) dose (800 mg/d) for 12-16 vs 24 weeks.
METHODS: We analyzed 142 CHC patients (22 with cirrhosis) who had been treated with PEG plus RIB for 24 weeks (group A, n=88) or with PEG plus RIB, both for 12-16 weeks (group B, n=39) or with PEG for 12-16 weeks plus RIB for 24 weeks (group C, n=15). SVR was confirmed by undetectable serum HCV-RNA at the end of treatment and again 24 weeks later.
RESULTS: Overall, 81.7% of patients exhibited SVR (group A-88.6%, group B-69.2% and group C-73.3%) and 7.08% were non-responders whereas 10.5% were relapsers. Non-SVR was significantly related with the treatment-group (p=0.026 for group B and p=0.002 for group C, compared to group A), the older patient's age (p=0.023) and the presence of cirrhosis (p<0.0001). In non-cirrhotic patients only the treatment-group (p=0.018 for group B and p=0.002 for group C, compared to group A) independently predicted the non-SVR. When we directly compared group B and group C we found no differences in the multivariate model that was adjusted for all the baseline parameters.
CONCLUSION: The SVR rates is independently predicted by the adherence to the combination treatment with pegylated interferon plus ribavirin for 24 weeks. The treatment duration with flat RIB dose in short-term PEG- treated patients didn't had any impact in the SVR rates.

PP-504

Transfer factor activation of the natural killer cell in patients with chronic hepatitis C infection

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Infection with hepatitis C virus (HCV) is a leading cause of chronic liver disease worldwide. Transfer factor advanced formula plus is formed of bovine and egg colostrum and has been found to increase NK cell activity by 437% above the base line. . AIM: Assessment of Natural Killer (NK) cell activation by transfer factor in patients with chronic HCV infection whom are not candidate for standard therapy. Material: 30 patients with chronic HCV infection whom are not candidate for standard therapy. Methods: (1) History and clinical examination. (2) Liver function tests. (3) Viral markers (HBS Ag, HCV Ab). (4) HCV RNA by PCR in the serum. (5) Flow cytometric analysis of NK cells in blood sample. (6) Patients were given transfer factor plus capsules 1x2 /daily before meals for 3 months then re-evaluation was done. Results: Significant reduction of ALT from (Mean ± SD 79.27 ± 71.47) to
(36.40 ± 3.23), AST from (80.73 ± 46.88) to (36.40 ± 3.23) and serum BiL from (1.58 ± 0.72) to (0.86 ± 0.33). Significant elevation of serum albumin from (3.19 ± 0.70) to (3.59 ± 0.54) and prothormbin activity from (0.63 ± 0.14) to (0.82 ± 0.12). No significant change in serum HCV RNA by PCR nor NK cell count by flow cytometry. Conclusion: Transfer factor advanced formula plus is an effective new therapeutic option for patients with chronic HCV infection whom are not candidate for standard therapy.

PP-505

Prevalence of Hepatitis C infection in hemodialysis patients in Rasht , Guilan, the North province of Iran

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Introduction: Hepatitis C virus (HCV) infection is a major health problem, as it can lead to chronic active hepatitis, liver cirrhosis, and hepatic carcinoma. Patients undergoing hemodialysis are at increased risk of HCV and other viral infections. Transmission of viral hepatitis, and in particular HCV through dialysis units, has shown a progressive increase worldwide. The aim of this study is to determine the prevalence of HCV infection in hemodialysis patients

Materials and methods: 163 patients from the hemodialysis unit of Rasht ,Guilan, the north province of Iran were interviewed and anti-HCV and was tested by a third-generation enzyme immunoblot assay (ELISA).SPSS 14 was used to analyze data. P>0.05 were considered statistically significant.

Results: The prevalence of HCV infection was 18.40%. Mean age in HCV^+ patients were 42.3± 11.2 years, 66.66% of whom were male. Duration of dialysis was 0-4 years in 33.33 % of HCV^+ patients, 4-8 years in 26.66% of cases, 8-12 years in 20% and 12-16years in 20% of them.

Conclusion: hepatitis C infection presents high prevalence in patients undergoing dialysis and anti-HCV test should be performed before admitting patients. Strategies such as closed control of services given to these patients like blood transfusion , training the personnel of hemodialysis units for preventing infection, isolation the units used by HCV^+ patients from other units are necessary and should be improved and need revised guidelines.

PP-506

Study of the relation ship between chronic hepatitis c infection, non alcoholic liver disease (NAFLD) and insulin resistance

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Background: Hepatic Steatosis (NAFLD) has been reported in 30 – 70 % of patients with HCV.

Aim of the work: Assess the relationship between Chronic Hepatitis C infection, NAFLD and Insulin resistance in Egyptian patients.

Material: 20 patients with Chronic HCV infection and hepatic steatosis were proven by liver biopsy were included in the study. Other 20 healthy adults were enrolled as a control group.

Methods: Liver function tests, HCV antibodies by third generation ELISA, HCV-RNA by PCR, fasting serum Glucose, fasting serum insulin and serum Ferritin. Insulin resistance was evaluated using the homeostasis model assessment (HOMA-IR) method. Also, ultrasound examination of the abdomen.

Results: Insulin resistance (IR) by (HOMA) index was significantly higher in patients than in control group (mean ± SD 8.36 ± 6.61 and 2.17 ± 0.08 respectively). Fasting Insulin level was significantly higher in chronic HCV patients than in control group (P = 0.000). Serum Ferritin level was significantly higher in patients (124.04 ± 123.18 ng/ml) than in control group (65.22 ± 46.27 ng/ml). HOMA-IR positively correlated with staging (P= 0.034, r= 0.477) and with grading (P=0.022, r= 0.509) in HCV infected patients.

Conclusion: HCV infected patients have high degree of insulin resistance (IR).

PP-507

Safety and activity of the HCV protease inhibitor TMC435350 in healthy volunteers, and chronic hepatitis C infected individuals: a phase I study

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Safety, tolerability and pharmacokinetics of TMC435350 were studied in HCV-negative volunteers, and efficacy was further assessed in chronic hepatitis C individuals.

TMC435350 as an oral solution in PEG400 was taken with food. The study involved single ascending (SAD) and multiple ascending (MAD) phases. HCV negative healthy volunteers were given single doses of 50-600mg TMC435350 or placebo, or 5 days dosing of 100-400mg once-daily (QD), or placebo. Six genotype 1 non-responders to prior IFN treatment received 200mg TMC435350 QD for 5 days. Subjects were monitored for safety, tolerability and pharmacokinetics. Plasma HCV RNA was measured using the Roche COBAS assay.

The healthy volunteer data have been reported (Verloes et al; Hepatology (2007) Vol 46, No 4, Suppl 1). No Grade 3 or higher AEs were observed in either HCV-negative or positive individuals. TMC435350 has a prolonged absorption with a t_{max} of 6 hours. Plasma concentrations were dose-proportional for single doses <100mg, but more than dose–proportional for higher single and repeated doses. Day 5 exposures in HCV+ were 3-fold higher than in HCV-negative subjects.

A rapid decline of HCV-RNA was observed. The median decline at Day 6 was 3.9 $\log_{10}$ IU/mL. No virologic breakthrough was observed during dosing. Sequencing revealed NS3 variants that were shown in vitro to be less sensitive to TMC435350, but remained sensitive to interferon. At 4 week follow-up, HCV plasma levels had returned to baseline levels.

TMC435350 was well tolerated during 5 days once-daily dosing and provoked a rapid antiviral activity in genotype 1subjects.

PP-508

The fitness of FibroTest in predicting fibrosis stage might not be influenced by the metabolic syndrome or occult HBV infection in Taiwanese chronic hepatitis C patients

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Background/Aims: The fitness of FibroTest for Taiwanese chronic hepatitis C (CH-C) patients, who have lower body mass index and higher prevalence of hepatitis B virus (HBV) infection, has not been investigated. We thus compared the predicting value of FibroTest with other non-invasive indices and analyzed the influence of metabolic syndrome or occult HBV infection over FibroTest.

Patients and methods: A total of 100 CH-C patients who were negative for HBsAg were enrolled prospectively to receive liver biopsy. FibroTest, APRI, and FIB-4 index were assessed and linked with the liver histology. The area under characteristic curve (AUC) for diagnosis of significant fibrosis (F2–F4) was compared in each index. The influence of metabolic syndrome and occult HBV infection was determined by its corresponding discordant rate of the fibrosis.

Results: The fibrosis stage (from F1 to F4) of the patients was 25%,

47%, 23% and 5% respectively. The AUC for diagnose significant fibrosis of FibroTest, APRI and FIB-4 were 0.65 (95% confidence interval: 0.53-0.77), 0.71 (0.61-0.82), and 0.65 (0.54-0.76), which didn't show significant difference. The prevalence of metabolic syndrome and occult HBV infection was 38.0% and 7.0% respectively. The overall discordant rate was 29.0%. The respective discordant rate in patients with/without metabolic syndrome was 34.2% and 25.8% respectively (P= .369). The individual discordant rate in patients with/without occult HBV infection was 0.0% and 31.2% (P= .104).
Conclusion: For Taiwanese chronic hepatitis C patients, the predictive value of FibroTest might not vary with the existence of metabolic syndrome or occult HBV infection.

PP-509

Treatment of hepatitis C associated severe mixed cryoglobulinemic syndrome with plasmapheresis improves tolerability and response to interferon-based therapy

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Introduction: Treatment of severe mixed cryoglobulinemic syndrome (MCS) associated with hepatitis C with interferon/ribavirin (IFN/RBV) is difficult due to renal dysfunction, vasculitis, and neuropathy. Methods: We reviewed the medical records of 23 patients (pts) who were diagnosed with hepatitis C associated severe MCS and received interferon-based therapy and/or plasma exchange (PE). Mean age was 54 years (46-63 years). 19 pts had HCV genotype 1. 15 pts had elevated HCV RNA; 11 pts had cirrhosis. 18 pts had elevated 24-hour urine protein; 5 pts had acute renal failure requiring hemodialysis (HD). 16 pts had vasculitic skin lesions; 9 pts had peripheral neuropathy. Treatment: Of 23 pts who received PE (mean: 8.2 treatments), 19 pts (83%) were candidates for IFN/RBV. 14 pts started Peg 2a/b IFN; 12 pts started RBV. 4 pts were not IFN/RBV candidates because of prior SVR or IFN/RBV failure. 11 pts received cyclophosphamide; 7 pts received rituximab. 8 pts required chronic PE. Result: 22 pts (96%) experienced clinical improvement. 10 of 14 pts with nephrotic proteinuria had >75% decline in proteinuria. 3 of 5 pts on HD stopped dialysis. 15 pts had improved vasculitic lesions; 7 pts had improved neuropathy. Of 14 pts who received IFN/RBV post-PE, 4 pts had SVR, 3 pts were NR, 1 pt was EVR (died), and 6 pts have ongoing treatment. SVR pts had marked improvement in MCS symptoms, whereas NR pts required chronic PE. Conclusion: Treatment of hepatitis C associated severe MCS with plasmapheresis improves tolerability and response to interferon-based therapy.

AUTHOR INDEX