Prevention of Liver Cancer

Kathryn Z. Guyton, PhD and Thomas W. Kensler, PhD

Address
Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205-2179, USA.
E-mail: tkensler@jhsph.edu

Current Oncology Reports 2002, **4:**464–470
Current Science Inc. ISSN 1523-3790

Hepatocellular carcinoma (HCC) is among the most prevalent and deadly cancers worldwide. Prominent risk factors for HCC include viral hepatitis infection; dietary exposure to hepatotoxic contaminants such as aflatoxins; alcoholism; smoking; and male gender. This review highlights ongoing efforts in HCC prevention. Strategies include vaccination against, and treatment of, viral hepatitis infection. In addition to interferon α, an acyclic retinoid (all-*trans*-3,7,11, 15-tetramethyl-2,4,6,10,14-hexadecapentanoic acid), glycyrrhizin and ginseng are currently under clinical investigation for HCC prevention in Japanese hepatitis C patients. Several recent clinical studies in a Chinese region of pervasive aflatoxin contamination also support the approach of favorably altering aflatoxin metabolism and excretion using the chemopreventive agents oltipraz or chlorophyllin. Agents exhibiting chemopreventive efficacy in preclinical HCC models include vitamins A, D, and E, herbal extracts, a 5α-reductase inhibitor, green tea, and *D*-limonene. Efforts to elucidate the molecular lesions and processes underlying HCC development have identified several putative molecular targets for preventive interventions. These include genes and gene products controlling viral replication, carcinogen metabolism, signal transduction, cell-cycle arrest, apoptosis, proliferation, and oxidative stress.

Introduction

Liver cancer is among the most common neoplasms worldwide, causing at least 500,000 deaths annually. The incidence of hepatocellular carcinoma (HCC) is especially high in southeast Asia and sub-Saharan Africa; however, HCC has recently increased in several low endemic areas including the United States, the United Kingdom, and western Europe, especially in men. The median age at presentation is 40 to 50 years, but HCC has been detected in patients as young as 6 years old [1]. HCC usually follows a rapidly progressive course and has a poor prognosis. Rates of intrahepatic recurrence exceed 25% per year following curative treatment, primarily because of underlying liver disease [2,3]. The 5-year mortality rate for HCC is 65% following surgical resection, and greater than 90% for the 80% of patients with nonresectable tumors. Screening programs have had little success in improving long-term survival.

Chronic liver disease is the predominant risk factor for HCC incidence and mortality. The most common cause of liver cirrhosis is hepatitis B virus (HBV) infection; an estimated 400 million people are infected with HBV, and 170 million are infected with hepatitis C virus (HCV) worldwide. Endemic viral hepatitis infection is indeed prevalent in areas of markedly high HCC incidence. For example, a prospective cohort study of 90,000 people in Haimen City, China identified HBV infection as the most prominent risk factor for HCC mortality in both males (relative risk [RR]=18.8; 95% CI, 15.7–22.5) and females (RR=33.5; 95% CI, 17.1–65.5) [4••]. HCV infection is perhaps more strongly associated with HCC, particularly in regions of relatively low HBV prevalence. In Japan, for example, approximately 16% and 80% of HCC cases are associated with HBV and HCV infection, respectively [5]. A separate prospective Japanese study found a fivefold-increased HCC risk for HCV compared with HBV [6]; about 20% of Japanese HCV patients are predicted to develop HCC in 10 years. In addition, coinfection with HBV and HCV has a synergistic effect on HCC risk. In areas such as Europe, where hepatitis virus prevalence is low, alcohol abuse is the leading risk factor for HCC. In a prospective study in southern Germany, chronic alcohol abuse was associated with nearly half (49.2%) of the 118 cases of HCC, whereas HCV infection was associated with only 17.8% [7]. Other factors that may act independently or synergistically with viral hepatitis to increase HCC risk include aflatoxin exposure and smoking [8].

Antiviral Approaches to Liver Cancer Prevention

HBV is a DNA virus that is readily transmitted perinatally; vertical transmission accounts for nearly half of HBV carriers in hyperendemic areas such as Korea and China. In contrast, HCV is an RNA virus that is primarily spread through blood transfusions and intravenous drug use. Development of an HCV vaccine has been complicated by the genetic heterogeneity of the virus [9,10]. However, HBV vaccination programs in Korea, China, and Taiwan have shown a protective efficacy of 90% to 95% and thus have considerable promise in HCC prevention. The neonatal

HBV vaccination program in Taiwan significantly reduced infant mortality from fulminant hepatitis [11]. Moreover, childhood HCC incidence decreased; the effect was significant for boys born in Taiwan after 1984, when the program was launched (RR=0.72; *P*=0.002) [12].

Treatment of viral hepatitis is another promising approach to HCC prevention. Approved antiviral therapeutic agents include immunomodulating agents (*eg*, interferon α and L(-)nucleoside analogues (*eg*, lamivudine). Lamivudine (3-thiacytidine) is tolerated well and produces sustained response in about 50% of patients [13]. However, development of clinical resistance conferred by mutations in the YMDD motif of HBV reverse transcriptase remains a major concern. In addition, a case of chronic HBV infection was reported in a newborn despite suppression of maternal HBV DNA to undetectable levels by lamivudine; this finding suggests continued risk of perinatal HBV transmission, even with successful antiviral therapy [14]. Several more potent L(-)nucleoside analogues under development may offer improved efficacy against HBV [15]. For example, β-L-2',3'-dideoxy-2',3'-didehydro-5-fluorocytidine was more efficacious than lamivudine in the woodchuck hepatitis virus model [16].

Interferon α, which produces sustained response in about 20% of HCV-infected patients, appears to reduce HCC risk by about threefold. In a prospective, randomized controlled study of 90 Japanese HCV patients followed for a mean of 8.7 years, HCC developed in 33 of 45 (75%) control subjects compared with 12 of 45 (27%) patients treated with interferon α [17••]. A retrospective analysis found HCC rates in untreated and interferon α–treated Japanese patients with HCV of 36.7% and 52.5%, respectively, at 10-year follow-up; among treated patients, a significantly greater protective effect of long-term (12 months or more) therapy with interferon α was found (*P*=0.0048) [18]. A greater protective effect with a dose of more than 500 million units has also been suggested in a Japanese retrospective analysis [19]. In keeping with these findings, a meta-analysis of 11 studies involving more than 2000 patients found significantly more frequent HCC development in control subjects (21.5%) compared with HCV patients treated with interferon α (8.2%; odds ratio [OR]=3.0; 95% CI, 2.3–3.9) [20••]. Interestingly, significantly more frequent HCC development was found in the five studies comparing control subjects with treated nonresponders (OR=2.7; 95% CI, 1.9–3.9). In these five studies, treated nonresponders developed HCC at a much higher rate than did responders (9% vs 0.9%; OR=3.7; 95% CI, 1.7–7.8). A retrospective multicenter analysis of 652 Japanese HCV patients treated with interferon α also found a significantly increased rate of HCC development in nonresponders compared with either sustained or incomplete responders (*P*<0.01) [21].

A meta-analysis of 18 trials involving over 4600 patients also found a protective effect of interferon α in HCV, but not in HBV patients [22••]. In those with HCV, risk differences among treated patients, sustained responders, or nonresponders versus untreated patients were -12.8% (*P*<0.0001), -19.1% (*P*<0.00001), and -11.8% (*P*<0.0001), respectively. Inconsistency among studies was significant and could only be countered by considering European reports alone; in this subgroup, risk differences with interferon α were not significant for HBV (-4.8%) and were less prominent for HCV (-10%, *P*<0.0001). Likewise, a study of 411 Chinese HBV patients found no protective effect with interferon α against HCC [23].

Clinical Liver Cancer Chemoprevention Studies

Several classes of chemopreventive agents have recently shown promise in populations at risk for HCC due to HCV infection. Possible mechanisms of such agents include antiviral and anti-inflammatory activities, as well as amelioration of liver damage and eradication of premalignant hepatic lesions. Glycyrrhizin, a licorice root extract that lacks HBV or HCV antiviral activity, is commonly used in Japan for HCV patients who have failed or did not receive interferon α. In a multicenter double-blind study, HCC developed significantly less frequently in 84 glycyrrhizin-treated Japanese HCV patients than in 109 control subjects (13% vs 25% at year 15; *P*<0.0002) [24]. An ongoing double-blind, randomized, placebo-controlled trial will test the efficacy of medicinal ginseng (1 g of red ginseng powder/d) in 300 Japanese HCV patients [25]. The endpoint of this multicenter trial is HCC development. Other agents being tested in Japanese HCV cohorts include Kampo herbal medicine [26]; continued study of acyclic retinoid (all-*trans*-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentanoic acid) [27], previously shown to reduce development of second primary hepatomas by about one third, is also planned.

Clinical interventions in Qidong, People's Republic of China, where aflatoxin contamination is pervasive and HCC is the leading cause of cancer death, have established the efficacy of oltipraz and chlorophyllin against aflatoxin biomarkers. These agents favorably affect the bioactivation and/or excretion of carcinogenic aflatoxin. Oltipraz (125 mg/d for 8 days) has recently been shown to transiently inhibit human CYP1A2 activity, as assessed by modulation of caffeine metabolism [28]. This finding is consistent with the observed 51% reduction in urinary excretion of the phase 1 metabolite aflatoxin M_1 following weekly administration of oltipraz (500 mg) in a phase IIa clinical study involving 234 adults in Qidong. Oltipraz is also known to potently and persistently induce phase 2 enzymes (particularly glutathione-*S*-transferases [GST]), a prominent effect in detoxification of aflatoxins. Among the enzymes induced by structurally related dithiolethiones is leukotriene B_4 12-hydroxydehydrogenase; this lipid-metabolizing enzyme was recently shown to possess a novel antioxidative activity that may contribute to

protection against aflatoxins and other carcinogens [29•]. A recent study using *nrf2*-deficient mice established an important role for the Nrf2 transcription factor in regulation of a number of protective phase 2 enzymes by dithiolethiones [30•].

Unlike oltipraz, chlorophyllin does not directly modulate the phase 1 and 2 metabolism of aflatoxin; instead, it functions as an interceptor molecule, forming complexes with aflatoxin and thereby diminishing carcinogen bioavailability. In a double-blind, placebo-controlled study in 180 adults in Qidong, chlorophyllin (100 mg 3 times a day for 4 months) reduced urinary aflatoxin-DNA adducts by 55% [31••]. Chlorophyllin has an improved safety profile compared with oltipraz, and follow-up clinical studies with this well-tolerated chemopreventive agent are warranted. Recent developments in aflatoxin biomarker assessment will facilitate such efforts. In particular, a liquid-chromatography electrospray–mass spectrometry assay of urinary aflatoxin biomarkers was recently validated in a rat aflatoxin chemoprevention model [32].

Chemopreventive Efficacy Studies in Preclinical Models of HCC

A number of additional agents have recently shown chemopreventive efficacy in preclinical models of liver cancer. The experimental protocols in which efficacy has been demonstrated vary widely with respect to inducing carcinogen, animal strain, and endpoints. The studies nonetheless suggest promising leads for clinical interventions provided that confirmatory preclinical efficacy and safety results are obtained. Effective agents include an organosulfur compound (*S*-methylcysteine), organoselenium compounds (ebselen, scordinin), herbal extracts (Picroliv, *Asteracantha longifolia* seed extract, *Salvia miltiorrhiza* extract), an antiandrogen (FK143, a 5α-reductase inhibitor), polyphenolic compounds (green tea), caffeine, *D*-limonene, and vitamins (α-tocopherol, 1α,25-dihydroxy vitamin D_3, vitamin A).

The chemopreventive efficacy of *S*-methylcysteine was suggested by significant decreases in the number and area of GST-P-positive foci induced by diethylnitrosamine (DEN) in male F344 rats [33]. GST-P-positive foci formation induced in male F344 rats by aflatoxin B_1 (AFB_1) was inhibited by the organoselenium compound ebselen [2-phenyl-1,2-benzisoselenazol-3(2*H*)-one] [34]; hepatic AFB_1-DNA adducts were likewise reduced. The garlic component scordinin significantly reduced DEN- and phenobarbitol-induced hepatic GST-P-positive foci, adenomas, and carcinomas in male F344 rats [35]. Herbal extracts with chemopreventive activity include Picroliv, an extract of *Picrorhiza kurroa*, which inhibited rat hepatic nodules induced by DEN and abolished the carcinogen-induced liver weight increase [36]. In male Wistar rats, an extract of *Asteracantha longifolia* seeds decreased the number and area of γ-glutamyl transpeptidase-positive foci initiated by DEN and promoted by 2-acetylaminofluorene (2-AAF) [37]. The traditional Chinese medicine *Salvia miltiorrhiza* significantly reduced both AFB_1-DNA adducts and GST-P-positive foci formation in male F344 rats [38]. In male F344 rats initiated with DEN and promoted by 2-AAF and hepatectomy, the 5α-reductase inhibitor FK143 significantly inhibited the formation of GST-P-positive foci as well as HCCs [39]. GST-P- and γ-glutamyl transpeptidase-positive foci in male F344 rats initiated by AFB_1 and promoted by CCl_4 were inhibited by green tea [40]. Notably, caffeine alone significantly reduced the incidence and multiplicity of hepatic adenomas and carcinomas in 2-AAF-treated ACI male rats in a dose-dependent manner [41]. *N*-nitrosomorpholine-induced GST-P-positive foci, neoplastic nodules, and HCCs were significantly reduced by *D*-limonene in male Sprague-Dawley rats [42]. Alpha-tocopherol substantially decreased the incidence of adenomas in DEN-treated transforming growth factor-α transgenic mice [43]. Vitamin D (1α,25-dihidroxyvitamin D_3) inhibited the formation of hepatic nodules initiated by DEN and promoted by phenobarbital in male Sprague-Dawley rats [44]. Finally, vitamin A as well as the retinoids all-*trans* and 9-*cis*-retinoic acid inhibited HCCs initiated by DEN and promoted by 2-AAF in male Wistar rats [45].

Molecular Lesions Underlying HCC Development

A number of molecular alterations that occur in HCC have been identified. One frequently mutated target in HCC is the *p53* gene. A specific missense mutation, a G-to-T transversion in *p53* codon 249, is associated with aflatoxin-induced human and experimental HCC. This mutation was detectable in the plasma of HCC patients by electrospray ionization mass spectrometry [46]. In some cases, the mutation was detected in plasma but not in tumor tissue, suggesting possible multiple independent HCCs. Other tumor suppressor genes that control cell-cycle arrest may also be commonly disrupted in HCC. A recent German study found disruption of the *p53* upstream regulators *p16* (INK4a) and *p14* (ARF) and/or *p53* mutation in 86% of 71 HCC cases [47]. Hypermethylation of the *p16* gene promoter, and the resultant loss of *p16* expression, was detected in the majority (64%, 30 of 47) of Japanese HCC cases, whereas 81% had either retinoblastoma protein or p16 loss [48]. A separate study also found p16 loss via hypermethylation in HCC (16 of 22 cases), as well as in five of 17 cirrhosis and four of 17 hepatitis cases [49].

Several recent studies have evaluated the modulating effects of regulators of hepatic proliferation and apoptosis on HCC. The *little* mutation, which confers growth hormone deficiency and thereby abrogates proliferation, suppressed DEN-driven HCC in mice [50]. On the other hand, increased proliferation without compensatory enhancement of apoptosis and accelerated HCC development was found in c-myc/$p53^{-/-}$ mice [51]. The viral protein HCV NS3 stimulated proliferation in NIH 3T3 cells, an

effect associated with *p53*-dependent repression of *p21* promoter activity [52]. Decreased expression of the apoptosis regulator *Bcl-x* was found in GST-P-positive foci and HCC initiated by DEN and promoted by phenobarbital and partial hepatectomy in a male F344 rats [53]. A member of the Bcl-2 family, the *Bcl-x* gene gives rise to two proteins: Bcl-xl, a dominant inhibitor of apoptotic cell death, and Bcl-xs, which promotes apoptosis. A Bcl-xs plasmid completely abrogated *N*-nitrosomorpholine-induced HCC formation in Sprague-Dawley rats [54]. Bcl-xs significantly increased apoptosis in hepatic nodules and foci in treated animals.

Genetic polymorphisms associated with enhanced susceptibility to hepatocarcinogens have also been identified in HCC. For example, the uridine 5'-diphosphate-glucuronosyltransferase UGT1A7*3 allele encodes an enzyme with low carcinogen detoxification activity. UGT1A7*3 was significantly associated with HCC in a German case–control study; 93.2% of 59 HCC patients had UGT1A7 polymorphisms, and 75% carried the UGT1A7*3 allele ($P<0.001$) [55•]. A statistically significant correlation between serum AFB_1-albumin adducts and HCC risk was found in a case–control study of Taiwanese HBV patients (OR=2.0; 95% CI, 1.1–3.7) [56•]. The effect of the aflatoxin biomarker on HCC risk was increasingly prominent among those with the GSTT1 null genotype (OR=3.7; 95% CI, 1.5–9.3, $P=0.03$). Interestingly, when the interaction between the biomarker and GSTT1 genotype was considered, aflatoxin exposure itself was not a significant risk determinant.

Oxidative DNA damage is elevated in the liver of patients with chronic liver disease and HCC. Levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) were significantly elevated in the peritumoral, compared with tumoral, tissue in 51 HCC patients ($P<0.005$) [57]. However, no differences in 8-OH-dG levels were found between tumor and surrounding normal liver tissue in 17 non-HCC patients. 8-OH-dG was also elevated in the liver of patients with chronic hepatitis, primary biliary cirrhosis, or alcoholism [58]. Hepatic oxidative stress was found in mice that were transgenic for the HCV core protein; the effect was exacerbated by alcohol but not associated with enhanced inflammation [59].

The role of DNA repair genes in HCC has been explored in several recent preclinical studies. For example, transgenic expression of human O^6-methylguanine-DNA transferase in the liver significantly protected C3HeB/FeJ mice from either spontaneous (*ie*, not carcinogen-driven) or *N*-methyl-*N*-nitrosourea-induced HCC development [60,61]. An opposite effect was found for deficiency in the xeroderma pigmentosum group A gene in C3H/HeN mice; deletion of this DNA repair gene significantly increased susceptibility to either spontaneous or AFB_1-induced HCC development [62].

The retinoid X receptor-α, (RXR-α), the most abundant retinoid receptor in the liver, is highly expressed in HCC. Mitogen-activated protein kinase (MAPK)-mediated phosphorylation of the accumulated RXR-α was associated with slowed degradation, low transactivating activity, and enhanced proliferation [63•]. Intriguingly, acyclic retinoid restored the transactivating function of phosphorylated RXR-α and promoted apoptosis in treated HCC cells [64]. Significantly higher expression levels of MAPK were found in HCCs than in surrounding normal tissue [65], suggesting one possible mechanism that may underlie enhanced RXR-α phosphorylation. A separate analysis showed that sustained activation of MAPK pathways was triggered by forced expression of the HBV X protein (HBx) in hepatocytes that are sensitive to transformation [66]. MAPK pathway activation was necessary for HBx-induced hepatocyte proliferation. HBx is a transcriptional transactivator implicated in HCC development, but its mechanism of action is unknown. Other potential mechanisms of HBx include disruption of intercellular adhesion [67]. This effect was associated with tyrosine phosphorylation of β-catenin, and it could be blocked by inhibition of src kinases. An analysis of HBx mutants suggests that COOH-terminal domains control proliferation and transformation by this viral protein [68].

Conclusions

HCC development is driven by multiple genetic and environmental factors that synergize to produce clinical disease. Molecular alterations associated with HCC development include *p53* loss of function mutations, *p16* gene hypermethylation, and polymorphisms in carcinogen detoxification genes. In addition, preclinical studies have identified a role for genes move that control apoptosis, growth factor–driven proliferation, oxidative stress, and DNA repair in this multifactorial disease process. Goals of HCC prevention efforts include elimination of risk factors, particularly viral hepatitis. Vaccination programs against HBV initiated in areas of high endemic infection in the 1980s are already showing success in HCC prevention. Although HCV vaccine development has been hindered by the genetic complexity of the virus, treatment of HCV-infected patients with interferon α reduces HCC risk. However, the magnitude of risk reduction is low (approximately threefold) and is strongest among the minority of patients (15%–25%) who achieve sustained response. Glycyrrhizin, commonly used in HCV patients who fail interferon α treatment, has suggested efficacy against HCC. Unfortunately, interferon α administered to patients with HBV-related cirrhosis appears to be without effect on HCC development. L(-)nucleoside analogues that are approved or under development for HBV treatment may hold promise in this regard. Several chemopreventive agents (*eg*, oltipraz and chlorophyllin) have recently proven efficacious in modulating important clinical biomarkers of aflatoxin metabolism and excretion. Preclinical chemopreventive efficacy studies have identified additional agents that may prove to be successful upon further preclinical and clinical testing against HCC development.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Teo EK, Fock KM: **Hepatocellular carcinoma: an Asian perspective.** *Dig Dis* 2001, **19:**263–268.
2. Koda M, Murawaki Y, Mitsuda A, *et al.*: **Predictive factors for intrahepatic recurrence after percutaneous ethanol injection therapy for small hepatocellular carcinoma.** *Cancer* 2000, **88:**529–537.
3. Bilimoria MM, Lauwers GY, Doherty DA, *et al.*: **Underlying liver disease, not tumor factors, predicts long-term survival after resection of hepatocellular carcinoma.** *Arch Surg* 2001, **136:**528–535.
4. •• Evans AA, Chen G, Ross EA, *et al.*: **Eight-year follow-up of the 90,000-person Haimen City cohort: I. Hepatocellular carcinoma mortality, risk factors, and gender differences.** *Cancer Epidemiol Biomarkers Prev* 2002, **11:**369–376.

This prospective cohort study of 58,545 men and 25,340 women identified HBV infection as the primary risk factor for HCC mortality in this Haimen City, China cohort.

5. Yoshizawa H: **Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future.** *Oncology* 2002, **62(suppl 1):**8–17.
6. Mori M, Hara M, Wada I, *et al.*: **Prospective study of hepatitis B and C viral infections, cigarette smoking, alcohol consumption, and other factors associated with hepatocellular carcinoma risk in Japan.** *Am J Epidemiol* 2000, **151:**131–139.
7. Hellerbrand C, Hartmann A, Richter G, *et al.*: **Hepatocellular carcinoma in southern Germany: epidemiological and clinicopathological characteristics and risk factors.** *Dig Dis* 2001, **19:**345–351.
8. Monto A, Wright TL: **The epidemiology and prevention of hepatocellular carcinoma.** *Semin Oncol* 2001, **28:**441–449.
9. Kao JH, Chen DS: **Recent updates in hepatitis vaccination and the prevention of hepatocellular carcinoma.** *Int J Cancer* 2002, **97:**269–271.
10. Prince AM, Shata MT: **Immunoprophylaxis of hepatitis C virus infection.** *Clin Liver Dis* 2001, **5:**1091–1103.
11. Kao JH, Hsu HM, Shau WY, *et al.*: **Universal hepatitis B vaccination and the decreased mortality from fulminant hepatitis in infants in Taiwan.** *J Pediatr* 2001, **139:**349–352.
12. Chang MH, Shau WY, Chen CJ, *et al.*: **Hepatitis B vaccination and hepatocellular carcinoma rates in boys and girls.** *JAMA* 2000, **284:**3040–3042.
13. Leung NW, Lai CL, Chang TT, *et al.*: **Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy.** *Hepatology* 2001, **33:**1527–1532.
14. Kazim SN, Wakil SM, Khan LA, *et al.*: **Vertical transmission of hepatitis B virus despite maternal lamivudine therapy.** *Lancet* 2002, **359:**1488–1489.
15. Cheng YC: **Potential use of antiviral L(-)nucleoside analogues for the prevention or treatment of viral associated cancers.** *Cancer Lett* 2001, **162(suppl):**S33–S37.
16. Le Guerhier F, Pichoud C, Jamard C, *et al.*: **Antiviral activity of beta-L-2',3'-dideoxy-2',3'-didehydro-5-fluorocytidine in woodchucks chronically infected with woodchuck hepatitis virus.** *Antimicrob Agents Chemother* 2001, **45:**1065–1077.
17. •• Nishiguchi S, Shiomi S, Nakatani S, *et al.*: **Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis.** *Lancet* 2001, **357:**196–197.

This prospective, randomized, controlled study of 90 chronic HCV patients found a protective effect of interferon α on HCC development and death (risk ratios vs symptomatic treatment, 0.256 and 0.135, respectively) after 8.7 years of follow-up.

18. Ikeda K, Saitoh S, Kobayashi M, *et al.*: **Long-term interferon therapy for 1 year or longer reduces the hepatocellular carcinogenesis rate in patients with liver cirrhosis caused by hepatitis C virus: a pilot study.** *J Gastroenterol Hepatol* 2001, **16:**406–415.
19. Toyoda H, Kumada T, Nakano S, *et al.*: **Effect of the dose and duration of interferon-alpha therapy on the incidence of hepatocellular carcinoma in noncirrhotic patients with a nonsustained response to interferon for chronic hepatitis C.** *Oncology* 2001, **61:**134–142.
20. •• Papatheodoridis GV, Papadimitropoulos VC, Hadziyannis SJ: **Effect of interferon therapy on the development of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis: a meta-analysis.** *Aliment Pharmacol Ther* 2001, **15:**689–698.

This high-quality meta-analysis, which considered 11 studies with 2178 HCV patients, found a significant protective effect of interferon α on HCC development. Although the effect was most pronounced for sustained responders, a reduced HCC incidence in nonresponders was also found.

21. Takimoto M, Ohkoshi S, Ichida T, *et al.*: **Interferon inhibits progression of liver fibrosis and reduces the risk of hepatocarcinogenesis in patients with chronic hepatitis C: a retrospective multicenter analysis of 652 patients.** *Dig Dis Sci* 2002, **47:**170–176.
22. •• Camma C, Giunta M, Andreone P, Craxi A: **Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach.** *J Hepatol* 2001, **34:**593–602.

This excellent meta-analysis considered three randomized and 15 nonrandomized controlled trials including 4614 patients and comparing interferon α treatment with no treatment. A protective effect of interferon α in HCV was identified, but the magnitude was low and related to response to interferon α. Interferon α did not affect HCC development in HBV-related cirrhosis.

23. Yuen MF, Hui CK, Cheng CC, *et al.*: **Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection: the effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications.** *Hepatology* 2001, **34:**139–145.
24. Kumada H: **Long-term treatment of chronic hepatitis C with glycyrrhizin (stronger neo-minophagen C [SNMC]) for preventing liver cirrhosis and hepatocellular carcinoma.** *Oncology* 2002, **62(suppl 1):**94–100.
25. The Ginseng-HCC Chemopreventive Study Osaka Group: **Study on chemoprevention of hepatocellular carcinoma by ginseng: an introduction to the protocol.** *J Korean Med Sci* 2001, **16(suppl):**S70–S74.
26. Cyong JC, Ki SM, Iijima K, *et al.*: **Clinical and pharmacological studies on liver diseases treated with Kampo herbal medicine.** *Am J Chin Med* 2000, **28:**351–360.
27. Okuno M, Kojima S, Moriwaki H: **Chemoprevention of hepatocellular carcinoma: concept, progress and perspectives.** *J Gastroenterol Hepatol* 2001, **16:**1329–1335.
28. Sofowora GG, Choo EF, Mayo G, *et al.*: **In vivo inhibition of human CYP1A2 activity by oltipraz.** *Cancer Chemother Pharmacol* 2001, **47:**505–510.
29. • Dick RA, Kwak MK, Sutter TR, Kensler TW: **Antioxidative function and substrate specificity of NAD(P)H-dependent alkenal/one oxidoreductase: a new role for leukotriene B4 12-hydroxydehydrogenase/15-oxoprostaglandin 13-reductase.** *J Biol Chem* 2001, **276:**40803–40810.

This study demonstrated a novel catalytic activity of NAD(P)H-dependent alkenal/one oxidoreductase (leukotriene $B_4$12-hydroxydehydrogenase): reduction of the α,β–carbon=carbon double bond of α,β–unsaturated aldehydes and ketones such as 4-hydroxy-2-nonenal. In addition, forced overexpression of the enzyme protected cells from the toxicity of 4-hydroxy-2-nonenal, a major product of lipid peroxidation.

30.• Kwak MK, Itoh K, Yamamoto M, *et al.*: **Role of transcription factor Nrf2 in the induction of hepatic phase 2 and antioxidative enzymes in vivo by the cancer chemoprotective agent, 3H-1, 2-dimethiole-3-thione.** *Mol Med* 2001, **7:**135–145.
This investigation characterized the expression patterns of over a dozen genes inducible by 3*H*-1,2-dithiole-3-thione in both wild-type and *nrf2*-disrupted mice. Nrf2 was found to play a central regulatory role in the constitutive and dithiolthione-inducible expression of multiple phase 2 and antioxidative enzymes.

31.•• Egner PA, Wang JB, Zhu YR, *et al.*: **Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer.** *Proc Natl Acad Sci U SA* 2001, **98:**14601–14606.
This double-blind, placebo-controlled study of chlorophyllin (100 mg three times a day for 4 months) in 180 residents of Qidong, People's Republic of China, demonstrated a 55% reduction in urinary aflatoxin-DNA adducts (P=0.036).

32. Walton M, Egner P, Scholl PF, *et al.*: **Liquid chromatography electrospray-mass spectrometry of urinary aflatoxin biomarkers: characterization and application to dosimetry and chemoprevention in rats.** *Chem Res Toxicol* 2001, **14:**919–926.

33. Fukushima S, Takada N, Wanibuchi H, *et al.*: **Suppression of chemical carcinogenesis by water-soluble organosulfur compounds.** *J Nutr* 2001, **131:**1049S–1053S.

34. Yang CF, Liu J, Wasser S, *et al.*: **Inhibition of ebselen on aflatoxin B(1)-induced hepatocarcinogenesis in Fischer 344 rats.** *Carcinogenesis* 2000, **21:**2237–2243.

35. Watanabe T, Sugie S, Okamoto K, *et al.*: **Chemopreventive effects of scordinin on diethylnitrosamine and phenobarbital-induced hepatocarcinogenesis in male F344 rats.** *Jpn J Cancer Res* 2001, **92:**603–609.

36. Rajeshkumar NV, Kuttan R: **Inhibition of N-nitrosodiethylamine-induced hepatocarcinogenesis by Picroliv.** *J Exp Clin Cancer Res* 2000, **19:**459–465.

37. Ahmed S, Rahman A, Mathur M, *et al.*: **Anti-tumor promoting activity of Asteracantha longifolia against experimental hepatocarcinogenesis in rats.** *Food Chem Toxicol* 2001, **39:**19–28.

38. Liu J, Yang CF, Wasser S, *et al.*: **Protection of salvia miltiorrhiza against aflatoxin-B1-induced hepatocarcinogenesis in Fischer 344 rats dual mechanisms involved.** *Life Sci* 2001, **69:**309–326.

39. Maruyama S, Nagasue N, Dhar DK, *et al.*: **Preventive effect of FK143, a 5alpha-reductase inhibitor, on chemical hepatocarcinogenesis in rats.** *Clin Cancer Res* 2001, **7:**2096–2104.

40. Qin G, Ning Y, Lotlikar PD: **Chemoprevention of aflatoxin B1-initiated and carbon tetrachloride-promoted hepatocarcinogenesis in the rat by green tea.** *Nutr Cancer* 2000, **38:**215–222.

41. Hosaka S, Kawa S, Aoki Y, *et al.*: **Hepatocarcinogenesis inhibition by caffeine in ACI rats treated with 2-acetylaminofluorene.** *Food Chem Toxicol* 2001, **39:**557–561.

42. Kaji I, Tatsuta M, Iishi H, *et al.*: **Inhibition by d-limonene of experimental hepatocarcinogenesis in Sprague-Dawley rats does not involve p21(ras) plasma membrane association.** *Int J Cancer* 2001, **93:**441–444.

43. Kakizaki S, Takagi H, Fukusato T, *et al.*: **Effect of alpha-tocopherol on hepatocarcinogenesis in transforming growth factor-alpha (TGF-alpha) transgenic mice treated with diethylnitrosamine.** *Int J Vitam Nutr Res* 2001, **71:**261–267.

44. Basak R, Bhattacharya R, Chatterjee M: **1 alpha,25-Dihydroxyvitamin D(3) inhibits rat liver ultrastructural changes in diethylnitrosamine-initiated and phenobarbital promoted rat hepatocarcinogenesis.** *J Cell Biochem* 2001, **81:**357–367.

45. Silveira ER, Naves MM, Vannucchi H, *et al.*: **Vitamin A and all-trans and 9-cis retinoic acids inhibit cell proliferation during the progression phase of hepatocarcinogenesis in Wistar rats.** *Nutr Cancer* 2001, **39:**244–251.

46. Jackson PE, Qian GS, Friesen MD, *et al.*: **Specific p53 mutations detected in plasma and tumors of hepatocellular carcinoma patients by electrospray ionization mass spectrometry.** *Cancer Res* 2001, **61:**33–35.

47. Tannapfel A, Busse C, Weinans L, *et al.*: **INK4a-ARF alterations and p53 mutations in hepatocellular carcinomas.** *Oncogene* 2001, **20:**7104–7109.

48. Azechi H, Nishida N, Fukuda Y, *et al.*: **Disruption of the p16/cyclin D1/retinoblastoma protein pathway in the majority of human hepatocellular carcinomas.** *Oncology* 2001, **60:**346–354.

49. Kaneto H, Sasaki S, Yamamoto H, *et al.*: **Detection of hypermethylation of the p16(INK4A) gene promoter in chronic hepatitis and cirrhosis associated with hepatitis B or C virus.** *Gut* 2001, **48:**372–377.

50. Bugni JM, Poole TM, Drinkwater NR: **The little mutation suppresses DEN-induced hepatocarcinogenesis in mice and abrogates genetic and hormonal modulation of susceptibility.** *Carcinogenesis* 2001, **22:**1853–1862.

51. Klocke R, Bartels T, Jennings G, *et al.*: **Lack of p53 accelerates hepatocarcinogenesis in transgenic mice constitutively overexpressing c-myc in the liver.** *FASEB J* 2001, **15:**1404–1406.

52. Kwun HJ, Jung EY, Ahn JY, *et al.*: **p53-dependent transcriptional repression of p21(waf1) by hepatitis C virus NS3.** *J Gen Virol* 2001, **82:**2235–2241.

53. Hatanaka Y, Nakae D, Mutai M, *et al.*: **Decreased expression of Bcl-x protein during hepatocarcinogenesis induced exogenously and endogenously in rats.** *Jpn J Cancer Res* 2001, **92:**1270–1277.

54. Baba M, Iishi H, Tatsuta M: **Transfer of bcl-xs plasmid is effective in preventing and inhibiting rat hepatocellular carcinoma induced by N-nitrosomorpholine.** *Gene Ther* 2001, **8:**1149–1156.

55.• Vogel A, Kneip S, Barut A, *et al.*: **Genetic link of hepatocellular carcinoma with polymorphisms of the UDP-glucuronosyltransferase UGT1A7 gene.** *Gastroenterology* 2001, **121:**1136–1144.
This German case–control study involved 59 cancer patients and 70 control subjects. The uridine 5'-diphosphate-glucuronosyltransferase allele UGT1A7*3, which encodes a protein with low carcinogen detoxification activity, was significantly associated with HCC.

56.• Sun CA, Wang LY, Chen CJ, *et al.*: **Genetic polymorphisms of glutathione S-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: a nested case-control study in Taiwan.** *Carcinogenesis* 2001, **22:**1289–1294.
This Taiwanese case–control study of chronic HBV carriers involved 79 HCC patients and 149 control subjects. A statistically significant correlation between HCC risk and serum AFB_1-albumin adducts was found. In addition, the effect of aflatoxin exposure on HCC risk was more pronounced among HBV carriers with the *GSTT1* null genotype (P=0.03).

57. Schwarz KB, Kew M, Klein A, *et al.*: **Increased hepatic oxidative DNA damage in patients with hepatocellular carcinoma.** *Dig Dis Sci* 2001, **46:**2173–2178.

58. Kitada T, Seki S, Iwai S, *et al.*: **In situ detection of oxidative DNA damage, 8-hydroxydeoxyguanosine, in chronic human liver disease.** *J Hepatol* 2001, **35:**613–618.

59. Moriya K, Nakagawa K, Santa T, *et al.*: **Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis.** *Cancer Res* 2001, **61:**4365–4370.

60. Zhou ZQ, Manguino D, Kewitt K, *et al.*: **Spontaneous hepatocellular carcinoma is reduced in transgenic mice overexpressing human O6-methylguanine-DNA methyltransferase.** *Proc Natl Acad Sci U S A* 2001, **98:**12566–12571.

61. Walter CA, Zhou ZQ, Manguino D, *et al.*: **Health span and life span in transgenic mice with modulated DNA repair.** *Ann NY Acad Sci* 2001, **928:**132–140.

62. Takahashi Y, Nakatsuru Y, Zhang S, *et al.*: **Enhanced spontaneous and aflatoxin-induced liver tumorigenesis in xeroderma pigmentosum group A gene-deficient mice.** *Carcinogenesis* 2002, **23:**627–633.

63.• Matsushima-Nishiwaki R, Okuno M, Adachi S, *et al.*: **Phosphorylation of retinoid X receptor alpha at serine 260 impairs its metabolism and function in human hepatocellular carcinoma.** *Cancer Res* 2001, **61:**7675–7682.

In surgically resected HCC and in HuH17 human HCC-derived cells, RXR-α was phosphorylated at serine 260 by MAPK. Phosphorylated RXR-α lost its transactivation activity, was resistant to degradation, and was associated with increased proliferation.

64. Okuno M, Sano T, Matsushima-Nishiwaki R, *et al.*: **Apoptosis induction by acyclic retinoid: a molecular basis of 'clonal deletion' therapy for hepatocellular carcinoma.** *Jpn J Clin Oncol* 2001, **31:**359–362.

65. Feng DY, Zheng H, Tan Y, Cheng RX: **Effect of phosphorylation of MAPK and Stat3 and expression of c-fos and c-jun proteins on hepatocarcinogenesis and their clinical significance.** *World J Gastroenterol* 2001, **7:**33–36.

66. Tarn C, Lee S, Hu Y, *et al.*: **Hepatitis B virus X protein differentially activates RAS-RAF-MAPK and JNK pathways in X-transforming versus non-transforming AML12 hepatocytes.** *J Biol Chem* 2001, **276:**34671–34680.

67. Lara-Pezzi E, Roche S, Andrisani OM, *et al.*: **The hepatitis B virus HBx protein induces adherens junction disruption in a src-dependent manner.** *Oncogene* 2001, **20:**3323–3331.

68. Tu H, Bonura C, Giannini C, *et al.*: **Biological impact of natural COOH-terminal deletions of hepatitis B virus X protein in hepatocellular carcinoma tissues.** *Cancer Res* 2001, **61:**7803–7810.