Hepatocellular Carcinoma: Updates in Primary Prevention

Will J. Fecht, Jr, MD and Alex S. Befeler, MD

Address
Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, 3635 Vista at Grand Avenue FDT 9S, St. Louis, MO 63110-0250, USA.
E-mail: fechtwj@slu.edu

Current Gastroenterology Reports 2004, **6:**37–43
Current Science Inc. ISSN 1522-8037

Hepatocellular carcinoma is a significant cause of mortality worldwide and a growing problem in the United States. Treatment options are often limited, and median survival is less than 1 year. Thus, prevention may provide the best opportunity to alter the natural history of this disease. Primary prevention is best exemplified by the successes of such public health measures as universal hepatitis B vaccination. Such antiviral therapies as interferon may also have a role. Lessons can be learned from complementary and alternative medicine. Nevertheless, more work is needed in understanding hepatocarcinogenesis and in developing models to assess potential chemopreventive agents.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, accounting for up to 1 million deaths annually [1•]. Although most of the worldwide burden of HCC comes from sub-Sahara Africa and Asia [2], its incidence is rising in the United States [3]. In 2003, the American Cancer Society estimated that 17,300 new cases and 14,400 deaths would occur from liver cancer in the United States [4]. Using several national databases, El-Serag and Mason [3] showed a 41% increase in mortality and a 70% increase in incidence comparing 1976 with 1980 and 1991 with 1995. In a more recent update, a further 25% increase in age-adjusted incidence rates from 1996 to 1998 was reported [5].

Hepatocarcinogenesis begins in the setting of chronic liver inflammation and hepatic fibrosis, often in association with hepatotrophic viruses or aflatoxin with increased expression of growth factors that lead to hepatocyte proliferation. Eventually, a series of genetic changes affecting oncogenes, tumor suppressor genes, and genes regulating cell growth, cell-cycle activity, mismatch repair, and apoptosis lead to the development of HCC [6•]. Many lines of evidence establish that hepatocarcinogenesis is strongly associated with the amount of fibrosis [6•]; more than 80% of patients have cirrhosis at diagnosis [7]. In the United States, the major risk factors for HCC include cirrhosis, infection with hepatitis C virus (HCV) and hepatitis B virus (HBV), heavy alcohol consumption, family history of HCC, male sex, older age, and African-American or Asian origin [8].

Survival after diagnosis is poor, especially when the disease is symptomatic, because of low potential for curative treatment and poor responses to noncurative medical therapy. Thus, the median survival is less than 1 year [2]. Furthermore, overall survival has not significantly improved during the past two decades [9]. Consequently, prevention may be the most effective means to improve outcomes.

Preventing the development of HCC is referred to as primary prevention. Fortunately, the natural history of HCC provides many opportunities for an impact to be made on the occurrence of primary liver cancer because of its known association with chronic liver disease and cirrhosis. The first opportunity involves the use of measures to avoid risk factors for HCC, including viral infections and toxins. This type of intervention includes public health programs that limit exposure to toxins or infections and vaccination. The second is early recognition and treatment of acute and inherited liver diseases, which may block the transition to chronic disease. The third is to prevent progression of chronic disease to cirrhosis. Finally, once cirrhosis is established, chemoprevention can interfere with the molecular events leading to HCC [10••]. Chemoprevention is usually defined as the use of artificial substances or dietary interventions to alter susceptibility to the actions of carcinogens and to retard, block, or reverse carcinogenesis [11]. Secondary prevention usually refers to early detection of established cancer and thus is really not prevention at all. Finally, tertiary prevention is defined as limiting HCC recurrence after initial curative treatment.

This review discusses hepatitis B virus, hepatitis C virus, alcohol, aflatoxin, and hereditary hemochromatosis (HH) with respect to primary preventive strategies, trends, and important developments. In addition, an update is provided on the progress of newer chemopreventive agents that suppress hepatocarcinogenesis in cirrhosis. Screening for HCC secondary and tertiary prevention are beyond the scope of this review.

Hepatitis B Virus

Hepatitis B virus is the most frequent underlying cause of HCC worldwide. In many developing countries where it is endemic, vertical and horizontal transmission in early childhood results in a high carriage rate because infections early in life are less likely to be cleared [12]. Conversely, in developed countries, such as the United States and Western Europe, sexual transmission and the use of injected drugs play a more prominent role, resulting in lower carrier rates. The risk of HBV-associated HCC is highest with actively replicating virus (usually greater than 10^5 copies/mL) and inflammation on liver biopsy, but it persists at a much lower rate in hepatitis B surface antigen (HBsAg) carriers with inactive liver biopsies. Even resolved infection, indicated by seroconversion to anti-HB surface (anti-HBs) antibody positivity is associated with increased risk of HCC, although to a much lesser extent [13].

Vaccination is the gold standard of primary prevention. Recombinant DNA-derived vaccines replaced plasma-derived forms in 1983 and have an excellent safety record, though the latter still dominate programs in developing countries because of lower costs. The success of the nationwide vaccination program in Taiwan, initiated in 1984, demonstrates the incredible impact of such an effort on prevention of chronic disease and primary cancer. By 1994, the rate of childhood HCC was significantly decreased [14]. Additionally, the carrier rate is expected to fall below 0.1% from 15% to 20% by 2010 [15]. Unfortunately, despite proven efficacy and cost-effectiveness, universal HBV vaccination has not been incorporated into many national immunization programs. Consequently, major efforts should be made to expand the availability of HBV vaccinations to endemic countries.

In the United States, selective immunization was replaced by universal immunization in early childhood or adolescence when the former did not affect the rising incidence of HBV infection. The vaccine, given at 0-, 1- and 6-month dosing intervals, confers 95% efficacy. Lower or incomplete seroconversion is observed in smokers, the elderly, and immunocompromised individuals [16]. Carriage in newborns is reduced by more than 90% when vaccine and passive immunoprophylaxis (within the first 48 hours of birth) are given to mothers with chronic HBV [17]. With respect to chronic liver disease, efficacy and safety have been established, although rates of seroconversion are diminished with cirrhosis. The authors recommend vaccination of all non-immune patients with other forms of chronic liver disease, before the development of cirrhosis if possible, to eliminate the increased risk of HCC and progressive liver disease from HBV infection.

Is booster immunization necessary? Approximately 50% of people vaccinated will lose anti-HBs within 5 years, although protection is not necessarily lost, because 90% will have an anamnestic response if given a booster [18]. Regular testing for anti-HBs in immunocompromised patients is recommended, with booster injection when titers fall below a "protective" (10 mIU/mL) antibody response [19].

When the window of opportunity to vaccinate is missed and chronic infection is established, antiviral therapy, including interferon (IFN) alfa and oral nucleoside analogues, has a potential role in prevention. In theory, antiviral agents could prevent HCC by direct chemopreventive effects on hepatocytes, elimination of chronic inflammation, and prevention of cirrhosis.

The preventive effects of IFN on the rate of carcinogenesis in cirrhosis have been examined in multiple nonrandomized controlled trials since 1992. Results from some studies suggest that treatment can improve survival and decrease the development of cancer. However, a meta-analysis by Camma *et al.* [20] failed to show reduction of HCC rates in the European studies with rigorous methodology. Likewise, in a systematic review, Baffis *et al.* [21] concluded that there is limited evidence for a preventive effect, but they point out that two studies show a strong reduction in risk of HCC in patients with a virologic response. Despite these disappointing results, use of IFN for hepatitis B is still indicated for its effects on reducing liver-related morbidity and mortality.

Lamivudine and adefovir, oral nucleoside analogues, can improve biochemical and histologic markers of chronic active HBV. In theory, the ability of these agents to improve fibrosis and potentially stop transition to cirrhosis makes them candidates for prevention of HCC. However, their role in prevention has not been adequately assessed by clinical trials and may never be assessed because of ethical concerns regarding withholding of a treatment that has proven efficacy against chronic hepatitis. Lamivudine may be inferior as a preventive agent because of the high rate of escape mutations, which appears to increase with each successive year of use. In clinical practice, lamivudine and adefovir should be used for their antiviral effects rather than for cancer prevention.

In addition to antiviral therapy, patient education about environmental influences is important. For example, in rural China, individuals who drank water from a ditch or pond were five times more likely to die from HCC than those using a river or deep well [12]. Similarly, public health measures to limit exposure to aflatoxin are important in China and Africa. Finally, limitation of alcohol consumption should be recommended, given its additive if not synergistic effects in hepatocarcinogenesis.

Hepatitis C

A large portion of the rise in incidence of HCC in the United States has been attributed to HCV infection. In the next 20 years, it is estimated that up to 186,300 new cases of HCV-associated HCC will be diagnosed [1•]. The major routes of transmission of HCV include injection drug use and blood transfusions prior to 1992. HCV accounts for more than 50% of HCC in the United States, Europe, and

Japan [13], in contrast to developing countries, where it is attributable to only 24% [22].

Primary prevention of HCV can be achieved by screening of blood products, adherence to universal precautions, and avoidance of injection drug use. The current risk of transmission of HCV is estimated to be less than 1/1,000,000/blood unit transfused [23]. Additionally, prudent though unproven recommendations include refraining from sharing razor blades and toothbrushes with known infected persons.

Symptomatic acute HCV infection can be treated effectively with a short course of IFN in 98% of patients, but the rate of spontaneous resolution of infection is high [24]. The authors recommend following quantitative HCV RNA with polymerase chain reaction (PCR) every month for 3 months after exposure. If the HCV RNA becomes undetectable in this time, HCV RNA by sensitive qualitative PCR should be used to confirm persistently undetectable virus. If the virus persists, treatment with IFN should be considered. Unfortunately, acute HCV infection rarely presents in the clinical setting.

An HCV vaccine would be ideal for primary prevention of HCC. Unfortunately, barriers to the development of a successful vaccine include the formation of quasispecies, broad variation of HCV protein sequences, ineffectiveness of neutralizing antibodies due to coating of the virion with host lipoproteins, and poor immunity after infection [16,18]. In addition, universal vaccination would likely be needed to establish an impact on HCV infection, as with HBV vaccination.

The evolution of chronic HCV treatment from IFN monotherapy to combination therapy with pegylated IFN plus ribavirin is a welcomed success in light of the difficulties in vaccine development. Sustained virologic and biochemical responses now occur in up to 56% of patients [25]. There is evidence that a sustained virologic response can lead to decreases in fibrosis and even reversal of cirrhosis [26]. Because HCV-associated HCC occurs almost exclusively in patients with cirrhosis, successful treatment with a sustained virologic response in patients without cirrhosis is likely to prevent future development of HCC [27].

Nevertheless, once cirrhosis has been established, a preventive benefit appears to be present with IFN monotherapy, especially in patients who achieve a sustained virologic response. In 2001, Camma *et al.* [20], in their meta-analysis of three randomized and 11 nonrandomized controlled trials, reported a low but statistically significant preventive benefit when their analysis was limited to studies with consistency. However, the benefit was mostly due to patients with a sustained response, who had a number needed to treat of 5. Methodologically sound studies to establish the preventive role of IFN in IFN-naïve HCV patients will never be done because random assignment to "no treatment" is unethical. Some authors have suggested that suppressive IFN therapy for pegylated IFN and ribavirin nonresponders is likely to have a preventive effect on HCC. Three large multicenter studies (HALT-C, COPILOT, and EPIC-3) address the use of suppressive pegylated IFN for HCV. This form of treatment is not recommended until trial results are available.

Hepatitis B and Hepatitis C Coinfection

It is not surprising that coinfection increases the risk of HCC, given the individual evidence for HBV and HCV infection. A meta-analysis of 32 epidemiologic studies demonstrated a synergistic but not additive effect of coinfection compared with HBV or HCV infection alone, with an odds ratio of 135 compared with 20 and 24, respectively [28]. These findings illustrate the importance of HBV preventive measures such as vaccination in the treatment of chronic HCV.

Alcohol

One in five individuals with chronic alcoholism, defined as more than 80 g of ethanol ingestion daily for 10 or more years, develops cirrhosis. Alcoholic cirrhosis is probably less likely than chronic hepatitis B or C to lead to HCC, although there are methodologic concerns regarding quantification of intake [12].

Concomitant alcohol ingestion in chronic viral hepatitis has been a subject of great investigational interest, yet conflicting results. A small case–control series in Italy revealed a progressive increase in the odds ratio for HCC development in patients with HCV-associated HCC and increasing alcohol consumption [29]. Conversely, a Japanese study of 3000 patients, of whom 22 developed HCC during the observational period, failed to identify an increased risk [30]. Nevertheless, there appears to be more evidence suggesting that alcohol exerts an additive if not synergistic effect on the incidence of HCC in chronic viral hepatitis.

Because evidence is lacking on what level of alcohol ingestion is safe in patients with chronic liver disease, some authors suggest complete abstinence [31]. However, small quantities of alcohol consumed on an infrequent basis are probably safe for most patients with liver disease. An appropriate history and physical examination are imperative to establish signs and symptoms of heavy alcohol consumption so that supportive interventions can be offered.

Aflatoxin

The consumption of natural carcinogens may cause HCC. Based on strong epidemiologic evidence, aflatoxins have been known to be hepatocarcinogenic since the 1960s, with the highest incidence in Asian countries. These fungal metabolites produced by *Aspergillus* species contaminate 25% of the world's food supply [17]. Developing countries with hot, humid conditions seem to be at greatest risk,

with oilseeds and cereal crops primarily affected. Although biomarkers for exposure exist, they are used mainly in the investigational setting [32].

Like exposure to alcohol, aflatoxin exposure appears to have a synergistic effect in chronic viral hepatitis. A case–control study of men who developed HCC in Shanghai, China revealed a relative risk of 59, compared with age- and neighborhood-related control subjects without markers for aflatoxin or chronic viral hepatitis B. The relative risks for aflatoxin metabolites and HBsAg alone were 3 and 7, respectively [33].

Prevention of aflatoxin-related disease should be addressed at the individual and community levels. Dietary modifications and crop harvesting techniques, such as engineering crop resistance, reducing crop stress, and improving storage are important ways of limiting aflatoxin exposure and should be embraced in countries where such exposure is endemic. Following a campaign of public education and awareness in China, a shift was seen from diets based on maize to diets based on rice despite the increased cost of the latter [17]. Unfortunately, in some countries resources are limited and exposure is unavoidable.

Advances in chemopreventive strategies may be effective at the individual level as a form of primary prevention of chronic aflatoxin exposure. Oltipraz, an antischistosomal drug, is a powerful inhibitor of hepatocarcinogenesis when given before and during carcinogen exposure in laboratory animals. Its preventive effects are believed to revolve around altered metabolic pathways of mycotoxins limiting formation of DNA adducts and elimination of toxic metabolites by increased urinary excretion after conjugation. In a phase IIa clinical trial performed in 1995 and published in 1999, healthy individuals were randomly assigned to receive 125 mg of oltipraz daily, 500 mg of oltipraz weekly, or placebo. High-dose oltipraz limited activation of aflatoxins, a necessary step in oncogenesis, whereas sustained lower-dose oltipraz induced conjugation, which is necessary for increased urinary excretion of toxic metabolites [34]. A 12-month phase IIb trial started in 1999 has not yet been published.

Hereditary Hemochromatosis

Of the inheritable metabolic liver diseases, the greatest opportunity for prevention of HCC is seen in hereditary hemochromatosis. Although 10% of Caucasians carry a single *HFE* gene mutation and homozygosity occurs in one in 250 individuals of Northern European descent, this disease often goes unrecognized until patients are symptomatic. At that time, interventions have less of an impact on morbidity and mortality [35].

Ample evidence in the literature suggests that iron depletion therapy prior to the onset of cirrhosis considerably reduces the incidence of primary cancer [36]. In addition, once cirrhosis has developed, the incidence of HCC is not altered, despite resolution of the iron-overloaded state. Two German prospective cohort studies following 259 and 163 patients with clinical, biochemical, and histologic evidence of HH demonstrated that HCC accounted for the majority of deaths: 27.5% (19 of 69) and 24.5% (13 of 53), respectively. In addition, all cancers developed in cirrhotic patients despite the remarkable observation that 79% (15 of 19) and 85% (11 of 13) achieved complete iron depletion [37,38]. In an Italian study by Fargion *et al.* [39] with 212 Italian patients, all reported deaths occurred in those with cirrhosis; HCC accounted for 45% of deaths, including patients who were iron depleted. However, the authors did not specify whether patients were iron depleted prior to development of cirrhosis. Although this evidence is indirect, it would be unethical to design a randomized, controlled trial examining the effects of phlebotomy on the incidence of HCC in patients identified with cirrhosis and iron overload.

Although HH-related HCC is rare in most large series, it may be a more powerful risk factor than HBV or HCV, especially in patients with established cirrhosis. Because detection and subsequent iron depletion can eliminate progression to cirrhosis, the importance of early diagnosis should be emphasized. In addition, it is prudent to take measures to limit other insults, such as alcohol and viral hepatitis.

Chemoprevention: Clinical Trials and New Frontiers

Potential chemopreventive agents will be given to a relatively healthy asymptomatic population of cirrhotic patients for many years. Thus, in addition to proven efficacy, they must be well tolerated clinically, safe for patients with impaired hepatic function, and devoid of even unusual severe adverse side effects. Potential strategies for chemoprevention include developing agents that either prevent transition to cirrhosis or limit hepatocyte susceptibility to epigenetic or genetic changes that lead to the development of HCC. These include nonspecific antifibrotic agents, agents that inhibit hepatocyte proliferation, agents that promote clonal deletion via apoptosis of premalignant hepatocytes, and agents that promote forced differentiation of premalignant hepatocytes to lower-risk phenotypes. A clear understanding of the molecular basis of hepatocarcinogenesis, in vitro and in vivo models of hepatocarcinogenesis, and clues from the epidemiology of HCC may suggest candidate substances for chemoprevention.

Once a potential agent emerges from the laboratory, the challenges of demonstrating effectiveness and safety are great. Using realistic estimates of incidence from an easily identified group of high-risk patients such as those with HCV-associated cirrhosis, over 5000 patients would be needed for a clinical trial to show a 25% reduction in HCC over 3 years. Thus, validated biomarkers or other new models that predict future development of HCC are needed. The National Cancer Institute recently funded a

network of investigators in various types of cancer including HCC to pursue phase I and II studies in chemoprevention. These researchers are actively working to develop a model to test chemopreventive agents for HCC. Once clinical effectiveness is demonstrated, cost-effectiveness may ultimately limit the utility of the agent overall or curtail its use to a small number of very high-risk patients. Despite the investigation and development still needed, some agents, mostly in the realm of complementary and alternative medications, are now in use and purported to be efficacious. Other agents have been suggested as good candidates for chemoprevention based on non-human data or proposed mechanism of action.

The most exciting news in chemoprevention of liver cancer came with the publication of the original study by Muto *et al.* [40] and subsequent follow-up of the acyclic retinoid, polyprenoic acid. In a randomized, controlled trial examining prevention of de novo HCC after curative treatment, these authors demonstrated significant reductions in new HCCs and prolonged survival. Although polyprenoic acid was used as a tertiary preventive agent, this study provides a model of primary prevention in order to circumvent the need for large numbers in a clinical trial [40]. Unfortunately, no further clinical trials have been published, and the medication is not available for use anywhere in the world. Several years ago one of the authors on this paper (Befeler) was unable to get access to this agent for a randomized trial to assess a new model for HCC chemoprevention. Nevertheless, many lines of evidence remain, including epidemiologic associations and antitumoral effects using in vitro and in vivo models of HCC, indicating that retinoids continue to be good candidates for chemoprevention.

Glycyrrhizin, marketed in Japan for 60 years as Stronger Neo-Minophagen C, is a naturally occurring extract of licorice root initially developed as an anti-allergy medication to treat dermatitis [41]. It is given as an intravenous infusion and is not currently available in the United States. This agent, which has potent anti-inflammatory activity, has been used since 1977 in Japanese patients with chronic HCV and currently in those who fail to respond to standard combination therapy. Its efficacy in improving aminotransferase elevation and histology scores has been shown in clinical trials [41]. No randomized, double-blind, controlled trials have explored the long-term preventive effects of this agent for HCC. Nevertheless, a retrospective cohort study using Cox proportional hazard modeling demonstrated that long-term intravenous administration of glycyrrhizin for HCV reduced the development of HCC by 2.49 times (95 % CI, 1.01–6.12) [42]. The side effect profile for glycyrrhizin was acceptable, but the intravenous administration probably precludes its broad use.

Sho-saiko-to (TJ-9), a Chinese herbal medicine composed of seven ingredients, with the most active component speculated to be *Scutellariae* species, prevents hepatic fibrosis while promoting fibrolysis in laboratory animals [43]. In an open-label, prospective, randomized, controlled study of patients with cirrhosis, a trend toward a lower cumulative incidence of HCC and survival was observed, compared with results from untreated control subjects; statistical significance was not achieved. However, the difference between the groups after 5 years was small, and no more recent trials have been published [44]. The authors do not recommend use of this agent until more studies are performed.

HOE 077, a prolyl 4-hydroxylase inhibitor, is a potential antifibrotic agent. In the choline-deficient l-amino acid diet rat model of cirrhosis and HCC, HOE 077 reduced the incidence of HCC from 90% to 50%. In addition, less fibrosis, manifested by a reduction in hydroxyproline, was observed [45]. In 1991 a single-dose study in healthy volunteers demonstrated similar kinetics and metabolites to those shown in animals [44], but no clinical studies in humans have been published to date.

Overexpression of cyclooxygenase-2 (COX-2) has been of investigational interest, particularly in the pathogenesis of colon cancer. The US Food and Drug Administration has approved celecoxib, a COX-2 inhibitor, for the reduction of polyps in familial adenomatous polyposis [46]. Similarly, overexpression has been observed in HCC and may be an early step in hepatocarcinogenesis based on high expression in nontumorous cirrhotic tissue and in well-differentiated tumors. Recent in vitro studies have demonstrated an ability to downregulate cell proliferation in human hepatoma cell lines, although the anti-HCC mechanisms have not yet been defined [47]. These preliminary data appear promising, but the use of COX-2 inhibitors may be difficult for patients with impaired coagulation and increased dependence on renal prostaglandins. Nevertheless, future in vivo studies may set the stage for clinical trials.

Curcumin, a spice and food-coloring agent, is a natural chemical derived from the root of the plant *Curcuma longa*. Based on multiple animal tissue studies, this agent possesses preventive effects against several cancers. Curcumin also has anti-inflammatory and beneficial metabolic effects. A study by Chuang *et al.* [48] demonstrated the preventive effect of curcumin in *N*-diethylnitrosamine–induced HCC formation in mice, with tumor rates decreasing from 100% to 38%. [48]. Based on Western blot analysis, it appears that curcumin blocks expression of proteins involved in cell proliferation, thereby promoting cell arrest or apoptosis. Further investigation is expected because phase I clinical trials revealed no adverse effects [49].

Limited preliminary evidence from animal and cell-line models of HCC indicates that sex hormone blockade, angiotensin-converting enzyme inhibitors may interfere with hepatocarcinogenesis.

Conclusions

Primary prevention may be the best means to alter the natural history of HCC, given the limited utility of therapeutic options and poor median survival. Most of the current success in the field is related to prevention of predisposing liver disease by such means as HBV vaccination. Treatments for liver disease, such as IFN for HCV and phlebotomy for HH, are likely to prevent HCC if the transition to cirrhosis is avoided. Potential agents from complementary and alternative medicine and laboratory models should be assessed in rigorous clinical trials. Unfortunately, proof of efficacy requires large expensive trials and might not be possible without development of appropriate biomarkers of HCC. It is hoped that the current revolution in molecular biology, including gene chip arrays and proteomics, will facilitate a better understanding of hepatocarcinogenesis and lead to the discovery of biomarkers and candidate substances for chemoprevention.

References and Recommended Reading

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

- • Of importance
- •• Of major importance

1.• Befeler AS, Di Bisceglie AM: **Hepatocellular carcinoma: diagnosis and treatment.** *Gastroenterology* 2002, **122:**1609–1619.

This article outlines recommendations for diagnosis and treatment of HCC.

2. Bosch FX, Ribes J, Borras J: **Epidemiology of primary liver cancer.** *Semin Liver Dis* 1999, **19:**271–285.
3. El-Serag HB, Mason AC: **Rising incidence of hepatocellular carcinoma in the United States.** *N Engl J Med* 1999, **340:**745–750.
4. Jemal A, Murray T, Samuels A, *et al.*: **Cancer statistics 2003.** *CA Cancer J Clin* 2003, **53:**5–26.
5. El-Serag HB, Davila J, Petersen N: **The incidence of hepatocellular carcinoma in the United States: Is it still rising?** *Gastroenterology* 2003, **124(Suppl 1):**A-690.
6.• Thorgeirsson SS, Grisham JW: **Molecular pathogenesis of human hepatocellular carcinoma.** *Nat Genet* 2002, **31:**339–46.

A compact up-to-date review of what is known about hepatocarcinogenesis. A better understanding of the mechanisms of HCC development is essential to progress in prevention of HCC.

7. Nzeako UC, Goodman ZD, Ishak KG: **Hepatocellular carcinoma in cirrhotic and noncirrhotic livers: a clinico-histopathologic study of 804 North American patients.** *Am J Clin Pathol* 1996, **105:**65–75.
8. El-Serag HB: **Epidemiology of hepatocellular carcinoma.** *Clin Liver Dis* 2001, **5:**87–107.
9. El-Serag HB, Mason AC, Key C: **Trends in survival of patients with hepatocellular carcinoma between 1977 and 1996 in United States.** *Hepatology* 2001, **33:**62–65.
10.•• Blum HE: **Molecular targets for prevention of hepatocellular carcinoma.** *Dig Dis* 2002, **20:**81–90.

This article provides links between our understanding of molecular hepatocarcinogenesis and potential prevention strategies.

11. Tamimi RM, Lagiou P, Adami HO *et al.*: **Prospects for chemoprevention of cancer.** *J Intern Med* 2002, **251:**286–300.
12. Monto A, Wright TL: **The epidemiology and prevention of hepatocellular carcinoma.** *Semin Oncol* 2001, **28:**441–449.
13. Yu MC, Yuan J, Ross RK, *et al.*: **Presence of antibodies to the hepatitis B surface antigen is associated with an excess risk for hepatocellular carcinoma among non-Asians in Los Angeles county, California.** *Hepatology* 1997, **25:**226–228.
14. Chang H, Chen C, Lai M, *et al.*: **Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children.** *N Engl J Med* 1997, **336:**1855–1859.
15. Huang K, Lin S: **Nationwide vaccination: a success story in Taiwan.** *Vaccine* 2000, **18(Suppl 1):**S34–S38.
16. Kao J, Chen D: **Recent updates in hepatitis vaccination and the prevention of hepatocellular carcinoma.** *Int J Cancer* 2002, **97:**269–271.
17. Wild CP, Hall AJ: **Primary prevention of hepatocellular carcinoma in developing countries.** *Mutat Res* 2000, **462:**381–393.
18. Prince A: **Prevention of liver cancer and cirrhosis by vaccine.** *Clin Lab Med* 1996, **16:**493–505.
19. Kane M, Banatvala J, Da Villa G, *et al.*: **Are booster immunizations needed for lifelong hepatitis B immunity?** *Lancet* 2000, **355:**561–565.
20. Camma C, Giunta M, Andreone P, *et al.*: **Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach.** *J Hepatol* 2001, **34:**593–602.
21. Baffis V, Shrier I, Sherker A, *et al.*: **Use of interferon for prevention of hepatocellular carcinoma in cirrhotic patients with hepatitis B or hepatitis C virus infection.** *Ann Intern Med* 1999, **131:**696–701.
22. Colombo M: **Hepatocellular carcinoma in patients with HCV.** *Baillieres Best Pract Res Clin Gastroenterol* 2000, **14:**327–329.
23. **Centers for Disease Control and Prevention.** http://ww.cdc.gov/ncidod/diseases/hepatitis/c/faq.htm#6b Accessed September 30, 2003.
24. Jaeckel E, Cornberg M, Wedemeyer H, *et al.*: **Treatment of acute hepatitis C with interferon alfa-2b.** *N Engl J Med* 2001, **345:**1452–1457.
25. Fried MW, Shiffman ML, Reddy KR, *et al.*: **Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection.** *N Engl J Med* 2002, **347:**975–982.
26. Shiratoi Y, Imazeki F, Moriyama M, *et al.*: **Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy.** *Ann Intern Med* 2000, **132:**517–524.
27. Di Bisceglie AM, Carithers RL, Gores GJ: **Hepatocellular carcinoma.** *Hepatology* 1998, **28:**1161–1165.
28. Donato F, Boffetta P, Puoti M: **A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma.** *Int J Cancer* 1998, **75:**347–54.
29. Tagger A, Donato F, Ribero ML, *et al.*: **Case-control study on hepatitis C virus (HCV) as a risk factor for hepatocellular carcinoma: the role of HCV genotypes and the synergism with hepatitis B virus and alcohol.** *Int J Cancer* 1999, **81:**695–699.
30. 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.
31. Riley TR, Smith JP: **Preventive care in chronic liver disease.** *J Gen Intern Med* 1999, **14:**699–704.
32. Yu S: **Primary prevention of hepatocellular carcinoma.** *J Gastroenterol Hepatol* 1995, **10:**674–682.
33. Ross RK, Yuan JM, Yu MC, *et al.*: **Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma.** *Lancet* 1992, **339:**943–946.
34. Wang J, Shen X, He X, *et al.*: **Protective alterations in phase 1 and 2 metabolism of aflatoxin b1 by oltipraz in resident of Qidong, People's Republic of China.** *J Natl Cancer Inst* 1999, **91:**347–354.
35. Bacon BR, Di Bisceglie AM: **Hereditary hemochromatosis.** In *Liver Disease: Diagnosis and Management.* Edited by Bacon BR. New York: Churchill Livingstone; 2000:140–149.
36. Plesch FN, Kubicka S, Manns MP: **Prevention of hepatocellular carcinoma in chronic liver disease: molecular markers and clinical implications.** *Dig Dis* 2001, **19:**338–344.
37. Niederau C, Fischer R, Purschel A, *et al.*: **Long-term survival in patients with hereditary hemochromatosis.** *Gastroenterology* 1996, **110:**1107–1119.

38. Niederau C, Fischer R, Sonnenberg A, *et al.*: **Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis.** *N Engl J Med* 1985, **313:**1256–1262.
39. Fargion S, Mandelli C, Piperno A, *et al.*: **Survival and prognostic factors in 212 Italian patients with genetic hemochromatosis.** *Hepatology* 1992, **15:**655–659.
40. Muto Y, Moriwaki H, Ninomiya M, *et al.*: **Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma: Hepatoma Prevention Study Group** *N Engl J Med* 1996, **334:**1561–1567.
41. 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.
42. Arase Y, Ikeda K, Murashima N, *et al.*: **The long term efficacy of glycyrrhizin in chronic hepatitis C patients.** *Cancer* 1997, **79:**1494–1500.
43. Okita K, Sakaida I, Hino K: **Current strategies for chemoprevention of hepatocellular carcinoma.** *Oncology* 2002, **62(Suppl 1):**24–28.
44. Oka H, Yamamoto S, Kuroki T, *et al.*: **Prospective study of chemoprevention of hepatocellular carcinoma with sho-saiko-to (TJ-9).** *Cancer* 1995, **76:**743–749.
45. Horn W: **General pharmacology, toxicology and future clinical development of HOE 077.** *J Hepatol* 1991, **13(Suppl 3):**S63–S65.
46. Steinbach G, Lynch PM, Phillips RK, *et al.*: **The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis.** *N Engl J Med* 2000, **342:**1946–1952.
47. Hu K: **Rationale and feasibility of chemoprevention of hepatocellular carcinoma by cyclooxygenase-2 inhibitors.** *J Lab Clin Med* 2002, **139:**234–243.
48. Chuang SE, Kuo ML, Hsu CH, *et al.*: **Curcumin-containing diet inhibits diethynitrosamine-induced murices hepatocarcinogenesis.** *Carcinogenesis* 2000, **21:**331–335.
49. Cheng AL, Hsu CH, Lin JK, *et al.*: **Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions.** *Anticancer Res* 2001, **21:**2895–2900.