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Lifestyle and Environmental Approaches for the Primary Prevention of Hepatocellular Carcinoma

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Synopsis / Abstract:

Patients with chronic liver disease are at increased risk of developing hepatocellular carcinoma (HCC). The majority of patients diagnosed with HCC have limited treatment options and a poor overall prognosis, with a 5-year survival that is less than 15%. Thus, preventing the development of HCC represents the most important and impactful strategy to improve patient outcomes. However, current guidelines lack specific recommendations for the primary prevention of HCC. Emerging data suggest that lifestyle factors – including diabetes, obesity, alcohol consumption and an unhealthy diet – play an increasingly central role in the pathogenesis of HCC, and that primary prevention strategies focused on lifestyle modification could represent an important approach to the prevention of HCC. Moreover, both experimental and epidemiological studies have identified promising chemopreventive agents for the primary prevention of HCC, including statins, aspirin and anti-diabetic drugs. However, additional research is needed to define the optimal approach to lifestyle modification that can achieve maximal HCC risk reduction.

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Cancer prevention; hepatocellular carcinoma; chemoprevention; lifestyle; modifiable risk factor

Introduction

Hepatocellular carcinoma (HCC) represents the third leading cause of cancer-related mortality worldwide, and is a major cause of death among patients with cirrhosis¹. In the United States, the incidence of HCC has tripled over the past 30 years, and mortality rates from HCC are increasing at an alarming pace^{2, 3}. Currently, it is recommended that patients at high risk for developing HCC undergo regular surveillance ultrasonography with assessment of alpha feto-protein (AFP)⁴. This approach has a sensitivity of 84% (95% CI 76–92) for the detection of any-stage HCC, however the sensitivity of ultrasound for detecting early-stage HCC is only 47% (95% CI 33–61)⁵. Moreover, the accuracy of ultrasound varies widely with body habitus and operator expertise⁶, and it is underutilized among high-risk populations⁷. Thus, HCC is often diagnosed at a late stage, when treatment options are limited and prognosis is poor⁸. Despite recent advances in treatment, patients diagnosed with HCC have a five-year survival rate of less than 15%, and 70% of patients experience tumor recurrence within five years^{1, 3}. Given these alarming trends, an urgent need remains to develop effective primary prevention strategies that improve patient outcomes by preventing the development of HCC.

HCC risk varies according to the underlying etiology of chronic liver disease, the severity of liver fibrosis, and individual clinical and demographic factors. Major risk factors for HCC include chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus (HCV) infection, alcohol-related liver disease and nonalcoholic fatty liver disease (NAFLD)^{9, 10}. The vast majority of HCC tumors arise within cirrhotic livers, however HCC may also arise in the absence of cirrhosis, particularly in patients with chronic HBV infection and NAFLD^{11–13}. There are also well-established disparities in the incidence of HCC, with the highest rates observed in men and in racial and ethnic minorities^{14, 15}. Additionally, HCC cases are often clustered in areas of high poverty and unemployment, relative to the general population¹⁶. Finally, an increasing body of literature now demonstrates that environmental and lifestyle factors play a key role in the pathogenesis of HCC, including diabetes, obesity, diet and use of certain medications^{17–21} (Figure 1). Thus, developing comprehensive strategies for HCC prevention requires a thorough assessment of risk, based upon these diverse clinical, demographic, lifestyle and environmental factors.

Given the limited treatment options and poor prognosis of HCC, strategies focused on preventing the development of HCC would likely carry the most impact. In this review, we outline recent advances in understanding of modifiable HCC risk factors, that could inform the development of much needed biomarker-based strategies for HCC prevention.

Overview of HCC Prevention Strategies

HCC prevention strategies can be applied before or during the natural history of chronic liver disease, and may be categorized as, primordial, primary, secondary and tertiary

prevention strategies. Primordial prevention includes behaviors and actions that maintain overall health and thereby prevent the development of risk factors for liver disease. Primary prevention is defined as the modification of behaviors or high-risk exposures in order to reduce risk factors for liver disease. Secondary and tertiary prevention includes screening and surveillance procedures that accurately identify and diagnose existing disease, facilitate early detection and timely interventions for HCC, or which minimize risk of HCC recurrence, among patients with established disease (Figure 2).

When this framework is applied to HCC prevention, primordial prevention involves maintenance of a healthy body weight, eating a healthy diet with minimization of alcohol use, vaccination against hepatitis B virus (HBV) infection, avoiding smoking, and maintaining normal circulating blood glucose and cholesterol levels. Primary prevention of HCC includes lifestyle and behavioral modification, including making changes to adopt a healthy diet, quit smoking or reduce alcohol consumption, weight loss, or taking medications to control or reduce risk factors, including diabetes, obesity, hypertension and/or dyslipidemia. Among patients with HBV or chronic hepatitis C virus (HCV) infection, the initiation of anti-viral therapy is also considered primary prevention, as the control of HBV DNA or the eradication of HCV infection can control these risk factors and thereby reduce long-term HCC risk. Finally, secondary prevention for patients with high-risk disease or cirrhosis includes engagement in regular HCC surveillance, every 6 months.

HCC risk can be reduced with etiology-specific treatments, which include the use of anti-viral therapy to suppress HBV DNA levels or to eradicate HCV infection, among those with chronic viral hepatitis. These etiology-specific strategies have been reviewed in detail elsewhere²². However, even with such therapies, excess HCC risk may nevertheless persist, particularly in high-risk patients or in those with cirrhosis^{9, 23}. Furthermore, as the prevalence of lifestyle-related liver diseases grows, it is increasingly recognized that primary prevention strategies focused on lifestyle modification are likely to provide the most impactful benefits^{11, 22}. However, to date, the optimal strategy for primary HCC prevention remains undefined.

Established and Emerging Lifestyle Risk Factors for HCC

Accumulating preclinical, clinical and epidemiological evidence demonstrate that modifiable environmental and lifestyle factors play a key role in the pathogenesis of HCC, including diet, alcohol use, obesity, type 2 diabetes and medications (Table 1). Accordingly, lifestyle modification has emerged as an important strategy for the primary prevention of HCC.

Alcohol

Alcohol use represents a major underlying etiology of HCC. Worldwide, approximately one-third of incident HCC cases are attributable to alcohol, although these rates vary markedly between regions^{24, 25}. For example, the proportion of HCC cases attributable to alcohol is estimated to be 6% in the Middle East, 14% in Northern Africa, 20% in southern Europe, and as high as 63% in some Eastern European countries²⁶. According to the Global Burden of Disease study, approximately 854,000 new primary liver cancers were diagnosed in 2015, and there were 815,000 liver cancer-related deaths²⁶. Of these recorded cases of new

primary liver cancer, 245,000 cases (30%) were attributable to alcohol use, with a strong male predominance (204,000 cases)²⁶. In longitudinal cohort studies from France, Spain, Belgium and Japan, the annual incidence of HCC among adults with alcoholic cirrhosis ranged between 2.1–5.6%^{27–30}. While prior studies have developed strategies to predict future HCC risk in this population^{27, 28}, large-scale validation studies are still needed. This represents a research area of important unmet need, for it is projected that the proportion of HCC cases attributable to alcohol is likely to increase in the coming decades, due to the improved efficacy of anti-viral therapies for chronic viral hepatitis, to HBV vaccination strategies, and to the increasing per capita consumption of alcohol that has been recorded in regions of Northern Europe, Eastern Europe, and in the United States^{24, 31}.

Epidemiological studies demonstrate that heavy alcohol use is independently associated with a 1.2-fold higher risk of developing incident HCC, compared to non-drinking³². In a meta-analysis of 19 cohorts and 4,445 incident cases of HCC, alcohol consumption contributed to HCC risk in a dose-dependent manner, with 46% higher risk observed with 50g of alcohol consumption/day, and 66% higher risk with 100g of alcohol consumption/day³². This excess risk is compounded in patients with underlying liver fibrosis^{24, 33, 34}, and alcohol contributes synergistically to the development of HCC in patients with obesity, diabetes^{33, 34}, and chronic HCV infection³⁴. Notably, even after alcohol cessation, the excess observed HCC risk related to alcohol use appears to last for many years³⁵. In a meta-analysis of 4 studies, HCC risk declined by approximately 6% per year with abstinence from alcohol; the authors found that for patients with cirrhosis, it would take approximately 23 years before an individual would achieve the same HCC incidence rates as a non-drinker³⁵.

NAFLD, obesity and diabetes

Worldwide, approximately 25% of adults are affected by nonalcoholic fatty liver disease (NAFLD)¹⁷. Closely linked to obesity and diabetes, NAFLD is thought to represent the hepatic manifestation of the metabolic syndrome. While the majority of patients with NAFLD have non-progressive disease, nearly 30% of adults with NAFLD develop nonalcoholic steatohepatitis (NASH) and fibrosis, and among those cases, between 10–20% progress to cirrhosis³⁶. NAFLD represents the most rapidly growing cause of cirrhosis in the United States, and it is also the fastest growing indication for liver transplantation, among adults with HCC³⁷.

Obesity and diabetes are present in 51% and 23% of patients with NAFLD¹⁷, and both conditions represent independent risk factors for the development of HCC. Epidemiological studies have linked excess adiposity (defined by, total body weight, body mass index [BMI], waist circumference, etc.) to an increased risk of incident HCC^{18–20}, and to a nearly 2-fold higher risk of HCC-related mortality³⁸. Similarly, type 2 diabetes is significantly and independently associated with excess HCC risk^{20, 39–42}. In a meta-analysis of 23 cohort studies, diabetes was associated with a 2-fold higher pooled relative risk of incident HCC³⁹. Furthermore, recent evidence shows that this risk increases with longer duration of type 2 diabetes⁴², with additional metabolic comorbidities^{32, 42}, and also that diabetes compounds HCC risk, among patients with chronic viral hepatitis⁴³.

There is increasing awareness of a link between NAFLD and HCC; however, clinical data are limited and conflicting regarding the precise magnitude of this risk. In a 2011 meta-analysis, the 5–10 year risk estimates of HCC incidence ranged from 0% to 38% and displayed marked heterogeneity, due to the small sample sizes and limited numbers of cases of incident HCC, among the included studies⁴⁴. This limitation was partially addressed by a 2018 retrospective cohort study of 296,707 U.S. Veterans with NAFLD and matched non-NAFLD controls, which found that a diagnosis of NAFLD was associated with a modest but statistically significant increased risk of developing HCC (incidence rate difference, 0.02 per 1000 person-years), and the highest excess risk was observed with NAFLD cirrhosis (incidence rate difference, 10.6 per 1000 person years)¹². However, this cohort was primarily male (94%), with NAFLD and cirrhosis identified by administrative codes and/or by surrogate serum fibrosis scores; thus, future studies are still needed in unselected, population-based cohorts, including those with NAFLD histology, to establish more precise and generalizable estimates of HCC risk across the complete NAFLD histological spectrum.

Recent evidence has also suggested that HCC risk might be elevated in patients with NAFLD who do not have cirrhosis⁴⁵. While prospective studies are still needed to fully define this relationship, it would suggest that the mechanisms that underpin NAFLD-related hepatocarcinogenesis may be less dependent upon liver fibrosis, compared to other etiologies of liver disease. It has been hypothesized that these mechanisms might relate to gut microbial dysbiosis, changes in circulating gut microbiota-derived metabolites (i.e. secondary bile acids or short chain fatty acids), oxidative stress, disruption of circadian rhythms, or dysregulation of circulating and hepatic adipokines and proinflammatory cytokines^{46–50}. Further research is needed in animal models and in human studies, to more precisely characterize these pathways and to translate these findings to novel preventive therapies.

Dietary Patterns

A growing body of clinical and epidemiological evidence suggests that dietary patterns may influence HCC risk. Dietary patterns reflect complex combinations of nutrients and individual compounds that act synergistically within whole foods and across combinations of foods to exert biological effects, which may impact long-term health outcomes⁵¹. In one of the earliest observational studies of dietary patterns and incident HCC risk, it was demonstrated that male and female participants in the Shanghai Men's and Women's Health Studies who adhered to a vegetable-based dietary pattern had a significantly reduced risk of developing incident HCC⁵².

More recently, the National Cancer Institute launched the Dietary Patterns Methods Project, which compares validated indices of overall diet quality in relation to incident cancers⁵³. These indices were selected based upon their established associations with cancer and cardiovascular disease⁵⁴, and include the Alternative Healthy Eating Index (AHEI), the Healthy Eating Index (HEI), the Dietary Approaches to Stop Hypertension (DASH), and the Alternate Mediterranean Diet (AMED). Since that time, three observational cohort studies have evaluated index-based dietary patterns in relation to HCC incidence^{21, 55, 56}. Two of those studies were conducted in three large, prospective U.S. cohort studies (the National

Institutes of Health/AARP Diet and Health Study, the Nurses' Health Study and the Health Professionals Follow-up Study), and found significantly lower HCC risk in participants with higher AHEI-2010 and AMED dietary scores^{21, 55}. The third study included 169,806 adults enrolled in the prospective Multiethnic Cohort study, and found that higher AMED dietary scores were associated with significantly lower risk of incident HCC (adjusted hazard ratio for the highest quintile vs. the lowest quintile, 0.68, 95% CI 0.51–0.90)⁵⁶. To date, however, published evidence is not yet sufficiently robust to recommend one particular diet for HCC primary prevention.

Individual Foods, Nutrients and Dietary Compounds

Fruit, Vegetables, Meat and Fat

Consumption of fruits and vegetables have also been studied in relation to HCC incidence. In a 2014 meta-analysis of 19 studies (1.29 million subjects and 3,912 cases of incident HCC), each 100g increase in daily vegetable intake was associated with an 8% lower risk of incident HCC, among the included cohort studies (OR 0.92, 95% CI=0.88–0.95)⁵⁷. In contrast, a null association was found for fruit consumption and incident HCC risk⁵⁷. Observational cohort studies and case-control studies have also evaluated the intake of red meat, white meat and fish, in relation to incident HCC. In a meta-analysis pooling results from 9 studies, the highest category of daily red meat intake was not significantly associated with increased HCC incidence, compared to the lowest category (pooled OR=1.10, 95% CI=0.85–1.42)⁵⁸. In contrast, both white meat and fish consumption were significantly associated with reduced HCC risk, when the highest vs. the lowest categories of consumption were compared (pooled OR for white meat and fish=0.69 [95% CI=0.58–0.81])⁵⁸.

Although human data regarding dietary fat intake and HCC risk are more limited, a notable study included 495,006 older adults enrolled in the prospective NIH-AARP cohort, and observed a higher daily intake of saturated fat at baseline was associated with a significant, 1.9-fold increased risk of incident HCC (HR 1.87, 95% CI=1.23–2.85)⁵⁹. In contrast, the prospective, European EPIC cohort study did not find a significant association between saturated fats and incident HCC risk (HR 1.08, 95% CI=0.88–1.34), while monounsaturated fats were inversely associated with HCC risk (per each 5g/day, HR 0.71, 95% CI=0.55–0.92)⁶⁰.

Coffee

Coffee contains well-described anti-inflammatory, antioxidant and antifibrotic properties, and it has been observed that coffee drinkers tend to have lower risk of developing advanced liver disease, including liver fibrosis⁶¹, cirrhosis and incident HCC^{62, 63}. Both the World Cancer Research Fund and the International Agency for Research on Cancer have also published reports supporting the beneficial effects of coffee for the prevention of HCC^{64, 65}.

A recent meta-analysis of 26 studies and 1,825 incident HCC cases demonstrated that consumption of at least 2 cups/day of coffee was associated with significantly reduced risk of incident HCC, with a pooled relative risk of 0.71⁶⁶. Per each additional 2 cups of coffee

consumed per day, the magnitude of observed benefit was significantly greater with caffeinated coffee (27% relative risk reduction) than with decaffeinated coffee (14% relative risk reduction)⁶⁶. Overall, the strength and consistency of the epidemiological associations for coffee has led to recommendations for moderate coffee consumption for HCC prevention in the 2018 guidelines from the European Association for the Study of the Liver (EASL)⁶⁷. However, several important questions remain unanswered, including the optimal “dose” and preparation of coffee (i.e. espresso vs. drip-coffee, type of coffee bean or roasting process), the optimal timing of initiation and the necessary duration of consumption during the natural history of liver disease, to achieve meaningful risk reduction. Thus, high-quality, prospective studies are needed in well-phenotyped populations, that include more specific details regarding coffee consumption.

Green Tea

Two meta-analyses have evaluated green tea consumption in relation to HCC incidence^{68, 69}. The most recent 2016 meta-analysis included 11 Asian cohort studies of more than 460,000 individuals and 3,694 cases of liver cancer, and demonstrated a pooled relative risk for incident HCC of 0.88 (95% CI=0.81–0.97), when the highest category of green tea intake was compared to the lowest category⁶⁸. In a dose-response analysis, the authors additionally found that each additional 1 cup of daily green tea was associated with a 3% reduction in HCC risk (95%CI=0.95–1.00). In contrast, data from European cohort studies has been mixed: in the European Prospective Investigation into Cancer and nutrition (EPIC) cohort, persons in the highest quintile of green tea consumption had a 59% lower risk of developing HCC (adjusted HR, 0.41 [95%CI=0.22–0.78]), compared to the lowest quintile⁷⁰ while two prior Italian case-control studies found null associations^{71, 72}.

Green tea is produced by heating or steaming fresh tea leaves at high temperatures, in processes that result in minimal oxidation and thus preservation of the polyphenols (i.e. catechins) within the tea. Between 50–75% of the primary catechins in green tea are epigallocatechin-3-gallate (EGCG), while the remainder include epigallocatechin, epicatechin-3-gallate (ECG), and epicatechin. In preclinical studies, EGCG inhibits carcinogenesis at numerous sites, including within the liver, albeit with potential risk of hepatotoxicity at high levels⁷³. However, in an epidemiological cohort study, higher levels of urinary catechins were associated with increased HCC risk, among subjects with positive HBV surface antigens, and this excess risk was magnified in patients with low circulating retinol levels (adjusted odds ratio, 2.62, 95%CI=1.25–5.51)⁷⁴. Given that green tea is the primary source of catechins, these data indicate that further research is needed to understand the relationship between green tea consumption and HCC risk, particularly among patients with chronic HBV infection.

Omega 3 Polyunsaturated Fatty Acids (PUFAs)

Preclinical data suggest that intake of the omega 3 (n-3) PUFAs, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), could prevent hepatocarcinogenesis by inhibiting the pro-inflammatory cyclooxygenase-2 (COX-2) enzyme, which in turn inhibits endogenous biosynthesis of prostaglandins and β -catenin signaling pathways⁷⁵, while simultaneously stimulating the endogenous biosynthesis of pro-

resolution lipid mediators. When fat-1 transgenic mice (which endogenously form n-3 PUFAs) were compared to wild-type pairs, both the size and number of hepatic tumors was reduced after diethylnitrosamine (DEN) treatment, and hepatic COX-2 expression was significantly reduced, while levels of circulating n-3 PUFA-derived pro-resolution lipid mediators were significantly elevated⁷⁶. These pro-resolution lipid mediators, which include lipoxins, resolvins, maresins and protectins, are also stimulated by aspirin, and have been shown in murine models to mediate anti-tumor activity (Figure 3)⁷⁷.

In a large, prospective cohort study of 90,296 Japanese adults consumption of an n-3 PUFA-rich diet and individual n-3 PUFA supplements were significantly and inversely associated with reduced HCC risk, in a dose-dependent manner⁷⁸. Specifically, compared to the lowest quintiles of n-3 PUFA intake, the adjusted hazard ratios in the highest quintiles were, 0.64 for n-3 PUFA-rich fish, 0.56 for EPA, 0.64 for DPA, and 0.56 for DHA⁷⁸. These findings are also supported by prior cohort studies that have similarly demonstrated significant, inverse associations between intake of diets rich in n-3 PUFA-rich fish or white meat, and reduced HCC risk^{79–81}.

Vitamin D

Both preclinical and clinical studies have linked higher levels of Vitamin D, 25(OH)D to reduced HCC incidence. In vitro, administration of 1- α ,25(OH)₂D has pro-apoptotic and antiproliferative effects on numerous cancer cells^{82–84}, and inhibits growth of HCC cell lines⁸⁵, by modulating cell cycle growth via induction of p21 and p27 tumor suppressor genes and suppression of cyclins and cyclin-dependent kinases^{86, 87}. In humans, higher vitamin D, 25(OH)D, levels have been associated with reduced HCC risk, with a relative risk of 0.51⁸⁸; in contrast, low 25(OH)D3 levels have been linked to excess HCC risk in patients with chronic HBV infection (adjusted hazard ratio 1.90)⁸⁹.

Several carcinogenic signaling pathways are hypothesized to be responsive to Vitamin D and its metabolites. First, 1- α ,25(OH)₂D has been shown to downregulate epidermal growth factor (EGF) receptor expression, which inhibits cell growth and promotes cell division, through mitogen-activated protein kinase (MAPK)-dependent pathways⁹⁰. Second, Vitamin D3 might inhibit VEGF-mediated endothelial cell proliferation and angiogenesis^{91, 92}. Finally, it has been posited that Vitamin D might act on insulin-like growth factor (IGF)-I and II signaling pathways⁹³, which may in turn impact liver cancer cell proliferation.

Branched Chain Amino Acids (BCAAs)

Preclinical data suggest that elevated circulating BCAA levels might protect against hepatocarcinogenesis. *In vivo*, BCAA treatment enhances mTOR signaling, which reduces both liver fibrosis and HCC^{94, 95}. In HCV-transgenic mice, BCAA administration reduces hepatic iron deposition and decreases reactive oxygen species (ROS) formation⁹⁶, and in high-fat diet-fed mice with NASH, BCAA therapy represses pro-fibrogenic gene expression in hepatic stellate cells and protects hepatocytes from apoptosis⁹⁷. Furthermore, *in vivo*, BCAA therapy suppresses expression of IL-6, IL-1b, IL-18 and tumor necrosis factor (TNF), reducing inflammation in both the liver and white adipose tissues, and inhibiting spontaneous HCC development⁹⁸.

BCAA therapy has historically been used as a treatment for hepatic encephalopathy, and clinical evidence linking BCAA supplementation to HCC incidence is sparse. In a prospective study of 299 Japanese patients with cirrhosis, those provided with branched-chain amino acid (BCAA) supplementation (5.5–12.0g/day) had a significantly lower risk of developing incident HCC (RR 0.45)⁹⁹, compared to controls. In a meta-analysis of 11 studies, oral BCAA supplementation in patients with established HCC was associated with improved mortality among Child-Pugh class B patients, and among those with higher levels of albumin (standardized mean difference=0.234), and lower rates of ascites (relative risk, 0.55)¹⁰⁰. More recently, in an observational study of 166 patients undergoing evaluation for liver transplantation, reduced plasma levels of valine and the valine to phenylalanine ratio were significantly associated with increased overall mortality¹⁰¹.

Other Dietary Compounds

Numerous additional dietary components and phytochemicals have been examined for their potential role in HCC chemoprevention, including curcumin, resveratrol, flavonoids (including silymarin), and carotenoids. However, to date, robust clinical evidence supporting HCC preventative effects from these compounds in humans are still lacking.

Statins

Statins, or 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are prescribed for the reduction of low-density lipoprotein (LDL) cholesterol levels. Beyond their cholesterol-lowering effects, statins also exert a diverse array of pleiotropic anti-inflammatory and anti-neoplastic effects. Both *in vitro* and *in vivo* studies demonstrate that statins block numerous carcinogenic signaling pathways, including those governed by Myc, Akt, integrins, Rho-dependent kinase, NF- κ B, interleukin-6 and the Hippo pathway, among others^{102–109}. By curtailing mevalonate synthesis, statins also inhibit downstream post-translational modification of Ras/Rho signalling proteins, which regulate cellular survival and growth, and they inhibit the cellular breakdown of p21 and p27, thereby permitting these molecules to exert potent growth-inhibitory effects^{106, 110}.

Statins also appear to exert direct anti-fibrotic actions within the liver, which may potentiate their anti-HCC benefits. Liver fibrosis is driven by the activation of hepatic stellate cells (HSCs), which undergo a phenotypic change from a quiescent state to become proliferative myofibroblasts. In preclinical studies, statins inhibit the activation and proliferation of HSCs^{111–113}, by upregulating Kruppel-like factor 2, a transcription factor that promotes HSC quiescence and thereby limits collagen production¹¹⁴. The administration of statins has also been shown to reduce pressures in the portal circulation, which may further limit hepatic fibrogenesis¹¹⁵, potentially through non-canonical Hedgehog signaling pathways¹¹⁶.

Observational studies largely support a link between statin use and reduced HCC risk. We observed a dose-dependent, inverse association between statin use and reduced risk of cirrhosis and incident HCC, among U.S. Veterans with chronic HCV infection¹¹⁷, and also in a post-hoc analysis of a randomized controlled trial¹¹⁸. Kaplan et al. studied a different cohort of U.S. veterans with new diagnoses of cirrhosis, and found significant HCC risk reduction with statin use for at least 90 days or more, compared to non-use¹¹⁹. These

findings have been confirmed in several large meta-analyses^{120–122}, which have demonstrated a significant, inverse association between statin use and reduced HCC risk. The observed benefits of statins were most apparent among observational studies, while in contrast, post-hoc analyses of prior randomized controlled trials (RCTs) of statins for cardiovascular disease have failed to demonstrate significant HCC risk reduction. Notably, however, those prior RCTs were not designed or powered to evaluate the long-term effect of statins on HCC incidence. Further, because they excluded patients with cirrhosis, they were comprised of study populations at very low risk for developing incident HCC^{120, 121}.

Emerging evidence further suggests that statin class may also influence HCC risk. Specifically, statins can be broadly divided into lipophilic and hydrophilic subclasses, and it has been hypothesized that lipophilic statins (i.e. atorvastatin, simvastatin, fluvastatin and lovastatin) may confer more potent anti-HCC effects than hydrophilic statins (i.e. pravastatin, rosuvastatin). This hypothesis based upon four lines of evidence. First, in preclinical studies, lipophilic statins suppress viral replication, potentiate antiviral therapy and stimulate anti-tumor immunity to a greater degree than is observed with hydrophilic statins^{123–126}. Second, in the setting of progressive liver fibrosis, the expression of organic anion transporter proteins on the surface of hepatocytes is markedly reduced, and this may prevent hydrophilic statins from entering hepatocytes, whereas lipophilic statins can passively diffuse across cell membranes^{127, 128}. Third, lipophilic statins limit cell growth and promote cellular apoptosis by inducing cell cycle arrest via regulation of Ras/Raf/MEK/ERK signaling¹⁰⁸. Further, administration of simvastatin to hepatocyte cell lines enhances expression of the proapoptotic BAX gene and suppresses expression of the antiapoptotic BCL-2 gene, indicating that lipophilic statins can induce apoptosis by acting at the pre-translational level, as well¹²⁹.

Epidemiological data comparing lipophilic and hydrophilic statins is both limited and conflicting. Specifically, two prior studies did not identify differences in HCC risk with use of lipophilic compared hydrophilic statins^{121, 130}; however, in a large, population-based cohort study of Swedish adults, we recently demonstrated significantly reduced HCC risk among lipophilic statin users, compared to non-users, while the relationship between hydrophilic statin use and incident HCC risk was null¹³¹. Although future studies are still needed to confirm these findings, such data suggest that the observed benefits previously associated with statin use in prior studies may have been driven by the unique, class-specific benefits of lipophilic statins.

To date, evidence supporting the use of statins for HCC chemoprevention is not yet sufficient to be incorporated into guidelines. First, published data derive primarily from observational studies, which lack the benefits of randomization, and are prone to selection or confounding by indication bias. Among prior studies, only a few have appropriately balanced the prevalence of underlying HCC risk factors (such as HBV and HCV infection, alcohol-related liver disease, diabetes, obesity and smoking status) between exposure groups^{119, 131}. Such imbalances could introduce confounding by indication, particularly because physicians historically have avoided prescribing statins to patients with liver disease out of concern for hepatotoxicity. Second, high-quality, prospective data are scarce regarding the optimal statin type, necessary duration of use, or the durability of statin-related treatment

response, nor is sufficient data available regarding the impact of statins in patients with NAFLD or alcohol-related liver disease. Finally, it remains unknown whether there might be potential additive benefits from the concomitant use of statins together with other medications with putative anti-HCC effects, such as aspirin or metformin. While some prior data suggests that the relationship between statin use and reduced HCC risk is not significantly modified by concurrent aspirin or antidiabetic medication use¹³¹, confirmatory studies are needed to validate these findings.

Aspirin

Preclinical evidence supports a role for aspirin in the prevention of HCC. Although the precise mechanisms remain undefined, both cyclooxygenase (COX)-dependent and COX-independent actions have been proposed. Specifically, the inducible, proinflammatory COX-2 enzyme is over-expressed in many cancers associated with obesity and chronic inflammation^{132, 133}, including HCC^{134, 135}, and aspirin irreversibly inhibits COX-2 expression in a dose-dependent manner¹³⁶. COX-2 expression in hepatocytes promotes the spontaneous development of HCC in mice, by reducing tet methylcytosine dioxygenase 1 (TET1) expression, silencing tumor suppressor genes and activating oncogenic pathways¹³⁴. Hepatocarcinogenesis has also been linked to hepatic translocation of two gut microbial metabolites, lipoleic acid and deoxycholic acid, which promote cellular senescence and upregulate COX-2 expression, driving the production of prostaglandin E2 and suppressing antitumor immunity⁴⁷. Moreover, by stimulating production of prostaglandins, COX-2 overexpression also promotes angiogenesis and cellular proliferation^{137–139}, by activating the pro-inflammatory protein kinase 3, mammalian target of rapamycin (mTOR) and nuclear factor κ B (NF κ B) signaling cascades¹³⁴. In contrast, aspirin inhibits NF κ B activation and protein kinase 3 signaling^{140–142}, and in preclinical models, pharmacologic inhibition of COX-2 or prostaglandin E2 prevents the proliferation of liver cancer cells¹³⁵ and promotes the resolution of liver fibrosis^{143–145}.

The benefits of aspirin in the liver may also derive from the inhibition of platelet activity, which has been shown to limit hepatic inflammation, fibrosis and hepatocarcinogenesis¹⁴⁶. Platelets play a central role in promoting accumulation of CD8⁺ T cells in the liver during chronic viral infection. They also generate platelet-derived growth factor-beta, which activates hepatic stellate cells and promotes fibrosis progression, in rodent models¹⁴⁷. Recently, Malehmir et al demonstrated in murine models that antiplatelet therapy with aspirin prevented the development of nonalcoholic steatohepatitis (NASH) and subsequent HCC via inhibition of platelet-derived glycoprotein 1b alpha, which subsequently reduced intrahepatic platelet accumulation, activation and immune cell trafficking¹⁴⁸. Together, these lines of evidence provide additional promising mechanistic explanations for the observed hepatoprotective effects of aspirin.

Clinical evidence regarding the impact of aspirin use on HCC incidence derives exclusively from observational studies. studies^{149–166}. Although some have reported conflicting results, most of these observational studies have found a significant, inverse association between aspirin use and reduced risk of incident HCC (Table 2). For example, within two prospective cohorts of U.S. women and men, we demonstrated that regular aspirin use was associated

with a significant, 49% lower risk of developing incident HCC (adjusted HR, 0.51, 95%CI 0.34–0.77), and these benefits were both dose- and duration-dependent¹⁶⁵. More recently, we confirmed these associations in a nationwide, unselected population of Swedish adults with chronic HBV or HCV infection, in whom low-dose aspirin use (<163mg) was associated with significant, duration-dependent reductions in risk of developing incident HCC (adjusted HR 0.69, 95%CI 0.62–0.76) and in the risk of liver-related mortality (adjusted HR 0.73, 95%CI 0.67–0.81)¹⁶⁶. Similarly, a pooled analysis of 10 US-based prospective cohorts (with nearly 1.1 million adults, and 679 incident HCC cases) reported a pooled HR for incident HCC of 0.68 with aspirin use, compared to non-use¹⁶⁴. Although these lines of evidence are promising, additional prospective data are still needed to more fully characterize the potential benefits of aspirin across the complete spectrum of chronic liver disease, and also to quantify the potential risks of bleeding associated with aspirin use.

Metformin

Anti-diabetic medications have also been explored as potential agents for HCC chemoprevention. Among them, the best studied is metformin, a biguanide derivative that blocks gluconeogenesis and enhances peripheral insulin sensitivity. Metformin exerts diverse anti-angiogenic, anti-inflammatory and anti-neoplastic effects; by activating AMPK, metformin inhibits hypoxia inducible factor 1 alpha and vascular endothelial growth factor signaling, which serve to block angiogenesis¹⁶⁷. Metformin also suppresses hepatic progenitor cell activation¹⁶⁸, and can inhibit cellular proliferation by suppressing NF-κB and reducing the expression of cyclin D1¹⁶⁷. Furthermore, in murine models, metformin prevents HSC activation and attenuates fibrosis¹⁶⁹, and it also appears to reduce HCC development, particularly when it is initiated before the development of cirrhosis¹⁶⁸.

In humans, several prior meta-analyses have demonstrated that metformin use is associated with reduced HCC incidence. The most recent meta-analysis included 19 studies and over 550,000 patients with diabetes, and found a 48% lower risk of incident HCC with metformin use, compared to non-use (pooled OR, 0.52, 95%CI=0.40–0.68)¹⁷⁰. Notably, the authors found no significant reduction in HCC incidence in a sub-analysis of 2 post-hoc studies of prior RCTs of metformin use among patients with diabetes (pooled OR=0.84 with metformin use vs. non-use [95%CI=0.10–6.83]); however, those 2 prior RCTs were limited by very few cases of liver cancer, and they were not designed or powered to assess HCC endpoints, thus their findings should be interpreted with caution¹⁷¹.

Pioglitazone, which stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma, has demonstrated efficacy for reducing liver fat and inflammation, in patients with established nonalcoholic steatohepatitis (NASH)¹⁷²; however, whether this translates to reduced HCC risk is still unknown. To date, one case-control study reported significantly reduced HCC risk with use of pioglitazone, compared to non-use (OR 0.83, 95%CI 0.72–0.95)¹⁷³, and two studies have found significant risk reduction with use of any thiazolidinedione medication, compared to non-use^{174, 175}, however others have demonstrated null associations^{176, 177}. Finally, although glucagon-like peptide-1 (GLP-1) receptor agonists have demonstrated short-term efficacy for the resolution of NASH¹⁷⁸, very

little is currently known about the long-term impact of GLP-1 receptor agonists or dipeptidyl peptidase-4 (DPP-4) inhibitors on HCC incidence.

Other Potential Chemopreventive Drugs

Although published data are limited, several other medications could represent plausible agents for HCC chemoprevention, including angiotensin converting enzyme (ACE) inhibitors and menopausal hormone therapy. The renin-angiotensin axis participates in liver fibrogenesis and hepatocarcinogenesis¹⁷⁹, and by activating NFkB, angiotensin II can promote the survival of hepatic myofibroblasts, but this effect is reversed with captopril treatment¹⁸⁰. Moreover, telmisartan, an antitension II type 1 receptor blocker (ARB), can prevent fibrosis and HCC development in rodents¹⁸¹. Finally, it is well-established that there are marked sex disparities in the incidence of HCC, with men being affected more frequently than women, leading to the hypothesis that estrogen may protect against HCC incidence. In support of this, a case-control study of 234 women with treated HCC and 282 healthy controls demonstrated that menopausal hormone therapy use was associated with reduced odds of developing HCC¹⁸². A meta-analysis of 87 studies also found that variants in the estrogen receptor 1 (ESR1) gene were associated with excess HCC risk¹⁸³. Moreover, in a large consortium of prospective U.S. cohort studies, bilateral oophorectomy was significantly associated with increased HCC incidence (HR=2.67, 95%CI=1.22–5.85), after accounting for other lifestyle and clinical factors and duration of exposure to exogenous hormone therapy^{184, 185}.

The Potential Impact of Lifestyle Modification for HCC Risk Reduction

Given the growing prevalence of chronic liver disease attributable to an unhealthy lifestyle, and the significant associations between high-risk lifestyle factors and excess HCC risk, HCC prevention strategies focused on adopting a low-risk lifestyle would likely offer substantial benefits. However, in order to identify priorities for public health interventions, it is important to quantify the magnitude of contribution of lifestyle factors to HCC risk. Using two nationwide, prospective U.S. cohort studies, we recently demonstrated that over 80% of HCC cases could theoretically have been prevented, with adherence to low-risk lifestyle. Such data underscore the enormous potential impact of primary HCC prevention efforts focused on lifestyle modification. Nevertheless, important knowledge gaps still remain. In order to translate such data into meaningful recommendations, well-designed, prospective studies are needed to define the optimal approaches to lifestyle modification to achieve clinically meaningful and durable HCC risk reduction, in patients who are at high risk of developing incident HCC.

Challenges and Future Directions

Progress in the development and clinical translation of HCC prevention strategies has thus far been limited by four important barriers. First, despite promising associations between low-risk lifestyle factors and reduced HCC risk, data are lacking regarding the optimal approaches to lifestyle modification that might translate to effective and durable HCC risk reduction, in high-risk populations. Second, the molecular mechanisms of

hepatocarcinogenesis remain largely uncharacterized^{11, 186}, due to the genetic heterogeneity of HCC tumors¹⁸⁷, and to suboptimal animal models¹⁸⁸, which limit the ability to translate hypotheses from preclinical studies to humans. Third, research into other cancers benefits from ready access to tumor biospecimens, precursor lesions and adjacent normal tissues, which facilitates the discovery and validation of targeted, molecular chemoprevention strategies¹⁸⁹. In contrast, access to HCC specimens is more difficult, as HCC tumors may be diagnosed without confirmatory pathological specimens. While there have been promising recent developments in molecular tools for HCC risk prediction, and in the use of liquid biopsy, the clinical utility of these approaches is not yet established¹¹.

Finally, a major challenge has been the need for large numbers of subjects and prolonged follow-up times, in HCC chemoprevention trials. It has been hypothesized that these requirements for large populations and prolonged follow-up are due to the inclusion of heterogeneous study populations, which dilute potential treatment effects. For example, two large chemoprevention trials of low-dose interferon therapy for patients with advanced fibrosis or cirrhosis failed to demonstrate significant HCC risk reduction with treatment^{189–191}. However, among patients with cirrhosis – the subgroup at highest risk of developing HCC – a significant treatment benefit was found. Thus, it is plausible that enrollment of an enriched, high-risk study population might maximize the potential to detect a treatment effect. This in turn would enable the design of more feasible and efficient clinical trials, requiring smaller numbers of patients and shorter follow-up times¹⁸⁶.

In addition to risk-stratified enrollment, biomarker-based HCC chemoprevention trials are needed. To achieve this goal, such biomarkers must, (1) predict future risk of HCC development, (2) predict response to chemoprevention therapy, and (3) provide insight into drug pharmacokinetics. Recently, molecular biomarkers of HCC risk have been developed and are undergoing rigorous validation for these purposes¹¹. For example, liver tissue-derived transcriptomic signatures have been validated for predicting incident HCC risk among patients with cirrhosis of any etiology, including chronic HBV or HCV infection, alcohol related liver disease, and NAFLD¹⁹². Based upon these results, enrollment was recently completed for a Phase I/II clinical trial of Erlotinib for the prevention of HCC in patients with cirrhosis ([NCT02273362](#)); this trial utilized a liver tissue transcriptomic prognostic signature as a selection factor for study enrollment and as a surrogate, biomarker-based endpoint. Demonstrating that a high-risk transcriptomic signature predicts meaningful HCC risk reduction with Erlotinib would form a strong scientific rationale for future biomarker-driven HCC chemoprevention trials that utilize molecular-based risk-stratified enrollment procedures and validated, surrogate biomarker endpoints for HCC¹⁸⁶. Such trials would have enhanced feasibility, overcoming many of the barriers outlined above, and would therefore enable more rapid translation of preclinical discoveries to humans.

Conclusions

Given the diversity of HCC and its underlying risk factors, strategies for primordial and primary HCC prevention are likely to have broad clinical applicability for patients with chronic liver disease. Lifestyle modification or the repurposing of medications like statins, aspirin or metformin, could be readily combined with etiology-specific HCC prevention

strategies, and might offer synergistic benefits. In parallel, research to better characterize the molecular determinants of HCC will help elucidate much needed prognostic biomarkers, and thereby enable the design of more efficient, biomarker-based HCC chemoprevention trials. Ultimately, combining lifestyle modification strategies with the use of safe, generic compounds and targeted biomarkers for predicting HCC risk could provide a robust and cost-effective strategy for HCC chemoprevention among at-risk patients with chronic liver disease.

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Key Points:

1. Cancer chemoprevention approaches can include primordial, primary or secondary prevention strategies; primary prevention strategies include modification of behaviors or high-risk exposures in order to eliminate risk factors for chronic liver disease.
2. Epidemiological data demonstrate that modifiable lifestyle factors contribute to the pathogenesis of HCC, including an unhealthy diet, alcohol use, obesity, type 2 diabetes and non-use of certain medications, including aspirin and statins.
3. Lifestyle modification or the repurposing of medications used for other conditions – including statins, aspirin or metformin – represent novel and important strategies for the primary prevention of HCC.
4. Research to define the molecular determinants of HCC could help elucidate much-needed prognostic biomarkers and thereby facilitate the design of more efficient, biomarker-driven HCC chemoprevention trials.

Figure 1.
Overview of HCC Prevention Strategies

Figure 2.
Emerging Risk Factors for Hepatocellular Carcinoma (HCC)

Figure 3.

Polyunsaturated fatty acids promote the endogeneous biosynthesis of anti-inflammatory, pro-resolution lipid mediators

Abbreviations: HEPE, hydroxyeicosapentaenoic acid; HpDHA, hydroperoxydocosahexaenoic acid; HDHA, hydroxydocosahexaenoic acid

Table 1.**Summary of Prior Studies Relating Lifestyle Factors with HCC Risk**

Risk Factor	Relative Risk Estimates*	Proposed mechanisms for HCC prevention
Obesity	HR 1.95, 95% CI 1.46–2.46 for body mass index (BMI) >30 (vs. BMI <25) ³⁸ ; HR 1.59, 95% CI 1.38–1.83 for elevated waist circumference (WC) vs. normal WC ¹⁹³	Hyperinsulinemia; lipotoxicity; adipokine disruption; alterations in the gut microbiome and gut-derived metabolites ^{47, 194–196}
Diabetes	RR 2.01, 95% CI 1.61–2.51 for diabetes (vs. no diabetes) ³⁹	Insulin resistance, hyperglycemia cause ROS formation, lipotoxicity and increased IGF-I and IGF-II levels, which activate Wnt signaling through PI3K/B-catenin pathways ^{197, 198}
Alcohol use	HR 1.16, 95% CI 1.01–1.34 for 3 or more drinks/day (vs. non-drinking) ³²	DNA adducts alter DNA repair mechanisms and change protein structure and function; induction of the CYP2E1 enzyme, mitochondrial dysfunction and ROS lead to cellular toxicity ¹⁹⁹
Diet	HR 0.68, 95% CI 0.51–0.90 for the highest quintile of the Alternative Mediterranean Diet (AMED) score (vs. the lowest quintile) ⁵⁶	Healthy diet reduces ROS formation and lipotoxicity, inhibits synthesis of pro-inflammatory cytokines and blocks B-catenin and COX-2 signaling pathways ^{75, 200}
Coffee	RR 0.71 for consumption of >2 cups of coffee/day (vs. none) ⁶⁶	Induction of UDP glucuronosyltransferase genes may have antioxidant and cytoprotective effects; caffeine inhibits CTGF and TGF- β ^{201, 202}
Aspirin	HR 0.69, 95% CI 0.62–0.76 for low dose (<163mg/day) aspirin use (vs. non-use) ¹⁶⁶	Inhibition of COX-2 and pro-inflammatory prostaglandins may reduce angiogenesis and tumor cell proliferation ¹³⁷ , by blocking protein kinase 3 and NFkB pathways ^{140, 141}
Statins	OR 0.63, 95% CI 0.52–0.76 for statin use (vs. non-use) ¹²⁰	Blockade of diverse carcinogenic pathways governed by Myc, PI3K-Akt, integrins, Rho-dependent kinase, NFkB, and the Hippo signaling pathway ^{102–109}
Metformin	OR 0.52, 95% CI 0.40–0.68 ¹⁷⁰ for metformin use (vs. non-use)	AMPK-mediated inhibition of VEGF and HIF1A, preventing angiogenesis and cell signaling ^{167, 203} , and suppression of hepatic progenitor cells ¹⁶⁸

Abbreviations: HCC, hepatocellular carcinoma; HR, hazard ratio; RR, relative risk; OR, odds ratio; CI, confidence interval; BMI, body mass index; WC, waist circumference; ROS, reactive oxygen species; COX-2, cyclooxygenase-2; CTGF, connective tissue growth factor; NFkB, nuclear factor kappa B; VEGF, vascular endothelial growth factor; HIF1A, hypoxia-inducible factor 1-alpha; AMPK, adenosine monophosphate-activated protein kinase

* Relative risk estimates were selected from meta-analyses (if available) or from the largest published cohort studies to date.

Table 2.

Observational studies of aspirin use and risk of hepatocellular carcinoma (HCC)

STUDY (AUTHOR, YEAR)	REGION	STUDY DESIGN	HCC CASES (N)	ASPIRIN USERS (N)	TOTAL (N)	HCC RISK (OR, RR, HR; 95% CI)
SIMON, 2020	Sweden	Retrospective Cohort	1,612	14,205	50,275	0.69 (0.62–0.76)
DU, 2019	China	Retrospective Cohort	41	59	264	0.16 (0.04–0.71)
LEE, T, 2019	Korea	Retrospective Cohort	697	2,123	10, 615	0.70 (0.58–0.86)
TSOI, 2019	Hong Kong	Retrospective Cohort	9,370	204,170	612,509	0.49 (0.45–0.53)
HWANG, 2018	Korea	Prospective Cohort	2,336	64,782	460,755	0.87 (0.77–0.98)
SIMON, 2018	USA	Prospective Cohort	108	58,855	133,371	0.51 (0.34–0.77)
LIN, 2018	Taiwan	Retrospective Cohort	110	3,576	18,243	0.67 (0.42–1.08)
TSENG, 2018	Taiwan	Retrospective Cohort	1,750	23,112	43,800	0.83 (0.69–0.99)
LEE, M, 2017	Korea	Retrospective Cohort [*]	63	343	14,392	0.34 (0.15–0.77)
LEE, T, 2017	Taiwan	Retrospective Cohort	NR	5,602	18,080	0.70 (0.37–1.36)
KIM, 2017	Korea	Case Control	229	390	1,374	0.34 (0.15–0.78)
YANG, 2016	UK	Case Control	1,195	1,670	5,835	1.11 (0.86–1.44)
PETRICK, 2015	USA	Prospective Cohort	679	477,470	1,084,133	0.68 (0.57–0.81)
SAHASRABUDDHE, 2012	USA	Prospective Cohort	250	89,585	300,504	0.51 (0.35–0.75)
CHIU, 2011	Taiwan	Case Control	1,166	162	2,332	1.0 (0.73–1.38)
FRIS, 2003	Danish	Retrospective Cohort	21	29,470	29,470	1.0 (0.60–1.50)
COOGAN, 2000	USA	Case Control	51	491	7,101	0.90 (0.30–2.90)

Abbreviations: N, number; HCC, hepatocellular carcinoma; OR, odds ratio; RR, relative risk; HR, hazard ratio; CI, confidence interval

^{*} Estimates provided are from the propensity score-matched cohort