Hepatocellular Carcinoma
It is a great pleasure and an honor to write the preface for this outstanding book dedicated to the therapy of hepatocellular carcinoma (HCC). This tumor, which is a major health problem worldwide, has stimulated the energy of several disciplines. The liver is a massive and complex organ requiring an excellent knowledge of its anatomy and physiology, with an exquisite comprehension of its impact on cardiovascular, pulmonary, and renal function.

Initially, the treatment of HCC was limited to surgical approaches with liver resection being the only option with curative intent. The development of HCC in chronic liver disease was associated with a high risk of technical difficulties and a high morbidity/mortality rate. This has challenged liver surgeons to improve their knowledge regarding liver anatomy, assessment of liver function, use of intraoperative imaging, tolerance of vascular clamping, and better anticipation of postoperative liver recovery. As shown in several chapters of this book, progress in liver surgery for HCC has expanded its development, yielding a true specialty. Imaging of liver parenchyma was motivated by two different goals including an efficient screening along with an accurate evaluation of the tumor in a background of abnormal parenchyma. Interventional radiology was initially focused on the treatment of this tumor with substantial technical advances allowing an efficient destruction of larger tumors. In parallel, radiologists developed transarterial chemoembolization and radioembolization. Those two locoregional approaches can stabilize the tumors, allowing in some cases for subsequent resection or transplantation. These multiple therapeutic approaches have contributed to expand the indications for liver transplantation, which remains the best curative treatment for limited HCC in patients with advanced chronic liver disease. However, this luxury treatment is restricted to very few countries, with a discrepancy between the increasing number of candidates and the limited number of grafts. Therefore, there is a considerable need for alternative treatments which are extensively developed in this textbook.

There is no efficient treatment without an accurate comprehension of the development of HCC. Beyond viral infections of the liver, the role of other potential causes is emphasized in an important chapter. There is no doubt that etiologies of HCC will not be considered similarly in the future, given more attention to both environmental and chronic medical conditions. These emerging factors, such as
metabolic syndrome, will probably highlight future targets relevant to the screening of high-risk patients.

The chapter on staging of HCC is very comprehensive. As such, J.N. Vauthey dedicated a great part of his initial studies to the stratification of patients with similar prognostic factors. The ongoing debate on transplant candidates confirms that stratification of patients is a prerequisite before considering any therapeutic modalities.

Indeed, our clinical experience shows that HCC is often a heterogeneous disease with variable outcomes. The chapter on pathologic considerations confirms that HCC has multiple histological components which will be clarified in the future by molecular classifications.

The last chapters of this remarkable book highlight that management of patients with HCC relies necessarily upon multidisciplinary effort involving the skills of radiologists, pathologists, oncologists, gastroenterologists, hepatologists, anesthesiologists, hepatobiliary, and transplant surgeons. In addition, these specific areas of knowledge and experience are guided by the important innovations from Asian countries and efficiency of medical treatment to an increasing degree. Of note in this book, supervised by eminent US authors, an entire chapter is devoted to the guidelines for treatment in Japan. Sorafenib has been approved as a standard of care for advanced HCC. Several studies evaluating other antiangiogenic agents and multi-target inhibitors are at various phases of their development with promising results. However, the most fascinating forthcoming issue will be the appropriate combination of medical treatment with surgical and radiological procedures. The improvement of resectability and survival observed in patients with colorectal liver metastasis treated by novel active chemotherapy was a major therapeutic step. Recent results in HCC strongly support that similar expectations might be achieved to improve outcome by including neoadjuvant or adjuvant therapy.

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October 2010
Preface

Hepatocellular carcinoma (HCC) is a major cause of cancer mortality worldwide. Because early detection is rare, the overall prognosis is generally poor. Understanding of the etiology, epidemiology, pathophysiology, molecular biology, and clinical features of HCC is important in providing optimal patient care. In addition, understanding of the limitations of our current knowledge and therapeutic capabilities is essential in order to guide future research efforts. Management of patients with HCC is necessarily a multidisciplinary effort which involves the skill of radiologists, pathologists, gastroenterologists, anesthesiologists, surgeons, medical oncologists, radiation oncologists, nurses, and other health professionals. This book is dedicated to the researchers, clinicians, and support staff involved in the fight against HCC, with admiration and appreciation for the work that is done every day to prevent, detect, and treat this disease. Most of all, this book is dedicated to the patients we treat, in the hope that sharing the collective wisdom of this esteemed group of experts will stimulate and encourage collaborative efforts to combat this formidable cancer.

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Liver cancer is the sixth most common cancer worldwide and the third most common cause of cancer mortality, with more than 500,000 deaths annually [1, 2]. Hepatocellular carcinoma (HCC), which comprises most primary liver cancer cases, is rarely detected early and is usually fatal within a few months of diagnosis [3]. A recently published study indicated that the incidence rates of HCC tripled in the United States from 1975 through 2005 [4].

Hepatocellular cancer has been shown to have wide variations in the geographic distribution, and there is a marked difference in the incidence between different races and genders. The highest incidence rates of HCC are in sub-Saharan Africa and Eastern Asia (>80% of all HCC), with China accounting for over 50% of the cases [5]. The low incidence countries include North and South America, Australia, and Northern Europe. HCC incidence varies among people of different ethnicity. For example, Chinese men have rates 2.7 times that of Indian men in Singapore [5]. In the United States, HCC rates are the highest in Asians, Hispanic, and African American middle-age men [4]. In most populations, the incidence of HCC is higher in males as compared to females. Surprisingly, the largest differences between the two genders are in the low-risk populations of central and southern Europe [6].

The peculiar pattern of HCC, that is the rise in the disease incidence among young persons and its varied incidence among different populations and races, suggests that this tumor is caused by several etiologic factors and that interactions among these factors may significantly increase the risk for HCC.

Many environmental and genetic factors have been identified as increasing one’s risk for the development of HCC. Furthermore, the synergy between these factors has been shown to be significant in hepatocarcinogenesis. This chapter reviews the
available data on these risk factors and generally discusses the pathogenesis of HCC development.

**Risk Factors of HCC**

*Hepatitis Virus Infection*

**Hepatitis B Virus**

The hepatitis B virus (HBV) genome is a partially double-stranded, circular DNA molecule. Since the identification of hepatitis B surface antigen (HBsAg) and its importance as a marker of chronic HBV infection, several epidemiological studies have established the significant hepatocarcinogenicity of chronic infection with HBV in humans; all were summarized by the International Agency for Research on Cancer (IARC) of the World Health Organizations (WHO) [7]. The association between HBV and HCC is not restricted to those who are positive for HBsAg; other studies have shown that some patients with hepatitis B core antibodies (anti-HBc)-positive and HBsAg-negative continue to be at risk for HCC development [8]. Meanwhile, after the initiation of HBV vaccination, significant declines in the incidence of HCC have been documented in high-risk countries like Taiwan [9].

The mechanism whereby HBV may induce HCC has been investigated through different approaches. The HBV-DNA integration has been detected in hepatocytes prior to tumor development among patients positive for HBsAg, which may enhance chromosomal instability and facilitate HCC development [10, 11]. In addition, the oncogenic role of the HBs and HBx proteins has been documented. HBx protein has been shown to transactivate both HBV and cellular genes, which may alter host gene expression and lead to HCC development [12]. In addition, the direct necrotic and inflammatory effect of viral hepatitis with cirrhosis cannot be excluded [13].

By using the complete nucleotide sequence of the viral genome, eight genotypes of HBV have been identified (A–H) [14]. The prevalence of HBV genotypes varies by geographical areas [15]. Genotype A is common in Europe, India, and Africa. Genotypes B and C are common in China, Japan, and Southeast Asia. Genotype D is common in Mediterranean areas and in the Middle East [16]. Genotypes E–G are common in Central and South America [15]. In the United States, all types are present with prevalence of 35, 22, 31, 10, and 2 for genotypes A, B, C, D, E–G, respectively [15]. A study showed that patients with genotype C infection may develop advanced liver disease rather than with genotype B or D. Genotype B was associated with hepatitis B e antigen (HBeAg) seroconversion at earlier age and less active hepatic inflammation. In addition genotypes A and B are associated with higher rate of HBeAg seroconversion during interferon therapy [17].

**Hepatitis C Virus**

Hepatitis C virus (HCV) is a small, single-stranded RNA virus [18]. The prevalence of HCV infection varies widely according to geographical areas. It represents
a major public health problem in the United States; approximately four million Americans are infected with HCV [19]. Several studies have demonstrated the significant role of HCV in the development of HCC. Antibodies against HCV (anti-HCV) can be detected in up to 90% of HCC patients [20]. A previously published meta-analysis of 21 case–control studies indicated that HCC risk was 17 times higher among HCV-positive individuals as compared to HCV-negative individuals [21]. HCV increases HCC risk by promoting progressive end-stage liver diseases. About 60–80% of anti-HCV-positive HCC patients were found to have liver cirrhosis [22].

It has been suggested that oxidative stress is one of the mechanisms involved in inflammation-related carcinogenesis in patients with chronic HCV infection [23]. In response to viral antigens, the activated macrophages and other recruited leukocytes release powerful reactive oxygen species (ROS) such as HOONO (from NO and $O_2^-$), HOCl, and $H_2O_2$, at sites of infection, causing areas of focal necrosis and compensatory cell division [24]. These oxidants not only kill target cells but may also overwhelm the antioxidant defenses of neighboring cells, leading to damage of important biomolecule, such as DNA, RNA, and proteins; if these relate to critical genes such as oncogenes or tumor suppressor genes, the initiation of cancer may result. In addition, ROS may serve as proinflammatory mediators [25].

Hepatocellular damage induced by oxidative stress may result in the recruitment of inflammatory cells and the activation of Kupffer cells and hepatic stellate cells (HSCs), which may enhance the inflammatory responses. Factors involved in this early phase are the release of proinflammatory and antiinflammatory cytokines [26, 27]. If oxidative stress persists, hepatic injury will also persist, and the activated HSCs will migrate and proliferate. As a consequence, extracellular matrix protein may accumulate in the damaged tissues, and the disease may progress to cirrhosis.

Like other RNA viruses, HCV displays a high genetic variability. On the basis of nucleotide sequence homology, whole-sequenced HCV isolates are classified as type I (1a), type II (1b), type III (2a), and type IV (2b). Provisionally, type V (3a) and type VI (3b) isolates were reported on the basis of data on partially sequenced genomes [28]. The geographic distribution of these genotypes demonstrated that genotypes I, II, and III are predominate in Western countries and the Far East, whereas type IV is predominant in the Middle East [29].

There is some evidence that the HCV genotype 1b is more aggressive and more closely associated with advanced chronic liver diseases such as liver cirrhosis and HCC [30, 31], although high prevalence of HCV type 1b has been reported among patients with HCC and no cirrhosis [32]. This information may indicate that in some cases the neoplastic transformation in type 1b infection may not require transition through the stage of cirrhosis. The observation that many HCC can develop in patients with HCV with no cirrhosis and that many of the HCV structural and non-structural proteins have not been entirely investigated indicates that the molecular mechanism of HCV in hepatocarcinogenesis is not well established.

Although HBV and HCV are the major etiologic factors for HCC development, approximately 60% of HCC patients are negative for HBV and HCV which implicates that other factors are involved (Fig. 1.1).
Environmental Risk Factors

Alcohol Consumption

Numerous studies included in a review by the international agency for research on cancer have concluded that alcohol consumption is an important risk factor for HCC development [33]. The alcohol–liver disease relationship correlates with the quantity of alcohol consumed over a drinking lifetime, with heavy alcohol consumption being the main risk for HCC and not social drinking [34]. Previous European studies [35, 36] reported a steep dose-dependent increase in relative risk of alcohol-induced liver disease above a “threshold” of 7–13 drinks per week in women and 14–27 drinks per week in men. Association between alcohol consumption and chronic liver diseases including HCC is partially related to ethanol metabolism and its major oxidation product, acetaldehyde [37], which modifies macromolecules in the cell by acetylation, leading to generation of free radicals, possible chromosomal abnormalities, and DNA mutation.

Our results from a US case–control study demonstrated approximately three-fold increase in HCC risk among individuals who consumed more than 60 ml ethanol per day [38]. The association between heavy alcohol consumption and HCC was larger in women than in men, which may be partially attributable to the synergism between female sex and heavy alcohol consumption. A recent review by Mancinelli et al. [39] suggested that women may experience a more rapid progression of alcohol damage than men. The lower body mass index and body fluid content in women than men may contribute to lowered ethanol diffusion and high blood concentration in women [40]. Moreover, the activity of gastric alcohol dehydrogenase, which
is responsible for the first-pass metabolism of ethanol in the stomach, is significantly lower in women than in men, which implies that large amounts of alcohol will be metabolized by hepatic alcohol dehydrogenase [41, 42]. It is also possible that genetic variations in carcinogen metabolism, inflammatory response, DNA repair, and cell cycle regulation play a role in determining individual susceptibility to alcohol carcinogenesis, which may partially explain variations in HCC risk by sex.

Seroepidemiological studies have demonstrated a high frequency of anti-HCV and HCV RNA in alcohol users and those among them who develop alcoholic liver diseases [43]. Despite this close relationship, there is little understanding of how HCV and alcohol may interact in the development of HCC. In most studies, anti-HCV in alcoholics was found to be closely associated with the presence of HCV RNA in serum, a marker of HCV replication [44], which may suggest that immunosuppression associated with chronic alcohol consumption may enhance HCV replication.

**Smoking**

Cigarette smoking is significantly associated with HCC development [45]. A meta-analysis on the association between smoking and liver cancer [46] concluded an overall OR of 1.6 (95% CI, 1.3–1.9) for current smokers and 1.5 (95% CI, 1.1–2.1) for former smokers. The recently released report by IARC had confirmed that smoking is considered a risk factor for liver cancer [47]. Despite evidence sufficient to judge the positive association between active smoking and liver cancer, smoking–HCC relationship in men and women separately has not been widely addressed. A US study suggested that smoking is more likely associated with HCC in men and not women [38]. Moreover, synergistic interactions between cigarette smoking and alcohol consumption, HBV, or HCV infection were reported by different studies [38, 48, 49]. Despite the significant association between cigarette smoking and the risk of HCC, passive smoking exposure is not associated with HCC development [38]. The use of chewing tobacco and snuff was also not related to HCC development in general or in nonsmokers [38].

The exact mechanism of tobacco hepatocarcinogenesis is unknown; however, of approximately 4,000 components identified in tobacco smoke, at least 55 are known carcinogens. The major chemical carcinogens include polycyclic aromatic hydrocarbons, such as benzo[a]pyrene; aromatic amines, such as 4-aminobiphenyl; and nitrosamines, such as 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone. A case–control study demonstrated that 4-aminobiphenyl DNA adducts contained in tobacco smoke is a liver carcinogen [50]. In addition, tobacco smoke contains volatile compounds (e.g., benzene), radioactive elements (e.g., polonium-210), and free radicals that may also play a role in hepatocarcinogenicity [51, 52]. Substantial evidence supports the notion that oxidative stress has been linked to tobacco use. In vitro studies demonstrated that the gas phase of cigarette smoke caused lipid peroxidation of human plasma, which was preventable by the addition of ascorbic acid.
This may support the smoking synergism with alcohol consumption and chronic viral hepatitis on HCC development.

**Aflatoxin Exposure**

Aflatoxins (AFs) are toxic secondary fungal metabolites (mycotoxins) produced by *Aspergillus flavus* and *A. parasiticus*. There are four AF compounds: B1, B2, G1, and G2 [55]. The most common and most toxic AF is AFB1, and the most important target organ is the liver, where the toxicity can lead to liver necrosis and bile duct proliferation [55].

In order for AFB1 to exert its toxic effects, it must be converted to its highly reactive 8,9-epoxide metabolite by the action of the mixed function monooxygenase enzyme systems in the liver (CYP450 dependent) [56, 57]. Therefore, the development of AF biomarkers is based on detection of the AFB1 active metabolites, which can covalently interact with cellular molecules, including DNA, RNA, and protein. Epidemiologic research has documented a significant risk for HCC development among individuals who consumed highly AF-contaminated diets [58, 59].

**Hormonal Intake**

The use of oral contraceptive pills and risk for HCC development is inconclusive. A recent review of 12 case–control studies that included 739 HCC cases and 5,223 controls [60] yielded an overall adjusted OR of 1.6 (0.9–2.5); however, six studies, included in the analysis, showed a significant increase in HCC risk with longer duration of exposure of oral contraceptives (>5 years). The observed association between liver cancer and oral contraceptive in animals is believed to be related to the proliferative effect of estrogen on hepatocytes where estrogen receptors exist and are highly expressed in HCC [61]. On the other hand, a protective effect of hormonal replacement therapy on liver cancer was determined by some studies [62, 63].

**Occupational Exposures**

Meta-analyses of epidemiological studies indicated a slightly increased risk of HCC with high level of occupation exposure to vinyl chloride [64]. However, such risk elevation can be a function of disease misclassification bias, since HCC was not analyzed separately from other liver tumors. Reviewing the epidemiological and experimental studies for the association between vinyl chloride and HCC indicated no evidence of biological plausibility for the risk of vinyl chloride on HCC [65].


**Chronic Medical Conditions**

**Diabetes Mellitus**

Because the liver plays a crucial role in glucose metabolism, it is not surprising that diabetes mellitus is an epiphenomenon of many chronic liver diseases such as chronic hepatitis, fatty liver, liver failure, and cirrhosis. A recent systematic review of several cohort and case–control studies concluded that diabetes mellitus is significantly associated with HCC [66].

There are several lines of evidence suggesting that diabetes is in fact an independent risk factor for HCC development. This evidence includes (1) results from review and meta-analysis reports concluding that diabetes is a risk factor of HCC [66–69]; (2) findings that the positive association between diabetes and HCC is independent from underlying cirrhosis and chronic liver diseases [70, 71]; (3) findings that the association is positively correlated with disease duration [72–74]; (4) demonstration of the synergistic interaction between diabetes and other HCC risk factors [72, 75, 76]; (5) findings of HCC recurrence after liver resection and transplantation among patients with diabetes [77, 78]; (6) suggestion of a biological plausibility that underlies the association between diabetes and HCC [67, 68, 79]; and (7) the observation of risk of HCC development among patients with type 1 diabetes mellitus [76].

The key mechanism for liver cell damage induced by type 2 diabetes mellitus involves insulin resistance and hyperinsulinemia [69, 80]. HCC development related to hyperinsulinemia can be mediated through inflammation, cellular proliferation, inhibition of apoptosis, and mutation of tumor suppressor genes [69]. Increased insulin levels lead to reduced liver synthesis and blood levels of insulin growth factor binding protein-1 (IGFBP-1), which may contribute to increased bioavailability of insulin-like growth factor-1 (IGF-1), the promotion of cellular proliferation, and the inhibition of apoptosis [81]. Insulin also binds to the insulin receptor and activates its intrinsic tyrosine kinase, leading to phosphorylation of insulin receptor substrate-1 (IRS-1) [82]. HCC tumor cells have been shown to overexpress both IGF-1 and IRS-1 [83]. Overexpression of IRS-1 has been associated with the prevention of apoptosis mediated by transforming growth factor-β [84]. In addition, insulin is associated with lipid peroxidation and increased oxidative stress and the generation of ROS, which may contribute to DNA mutation [85].

**Obesity**

It is well established that obesity is significantly associated with a wide spectrum of hepatobiliary diseases, including fatty liver diseases, steatosis, and cryptogenic cirrhosis [68, 86]. Once steatosis has developed, cellular adaptations may occur to allow the cell to survive in the new stressful environment and enhance vulnerability to a second hit, or genetic and environmental factors, leading to necroinflammatory changes (non-alcoholic steatohepatitis) or non-alcoholic steatohepatitis (NASH) where different mediators are involved in such pathogenesis [87]. However, there
is little information regarding the association between obesity and HCC. A recent meta-analysis 11 cohort studies reported a summary relative risks (95% CI) of 1.17 (1.02–1.34) and 1.89 (1.51–2.36) for overweight and obese individuals, respectively [88]. Nevertheless, the study did not separate HCC from other primary tumors of the liver nor control for the confounding effect of HCV, HBV, diabetes, and heavy alcohol consumption on HCC development.

Lipid peroxidation and free oxygen radicals may play a central role in NASH during which the initiation stage of HCC mechanism takes place. Proliferation of oval cells (the cells of origin for several types of liver cancer) and mutation of P53 tumor suppressor gene can also be potentiated. It is then suggested that the second stage (promotion) takes place as a result of balance in apoptotic and antiapoptotic factors; disturbance in growth factors such as TNF and TGF may facilitate oval cell proliferation [89]. Progression to HCC (stage 3) is suggested to be mediated through cyclooxygenase-2 (COX-2) gene expression by peroxisome proliferator-activated receptor (PPAR-β) nuclear receptors implicated in fatty acid oxidation, cell differentiation, inflammation, cell motility, and cell growth [90, 91]. It was suggested that PPAR-β promotes human HCC cell growth through induction of COX-2 expression and prostaglandins (PGE2) synthesis. The produced PGE2 phosphorylates and activates cytosolic phospholipase A2α (cPLA2α), releasing arachidonic acid for further PPAR-β activation and PGE2 synthesis via COX-2. This positive-forward loop between PPAR-β and PG pathway likely plays role in the regulation of human cell growth and HCC development (Fig. 1.2).

Fig. 1.2 Steps in hepatocarcinogenesis, modified from Xu et al. [90] and Bensinger and Tontonoz [91]

On the other hand, the association between obesity and HCC is hammered by the following obstacles: (1) categorizing HCC among patients with primary liver cancer, (2) inappropriate adjustment for the confounding effect of HCC risk factors specially type 2 diabetes mellitus, and (3) misclassification of obesity definition among patients with HCC. Relying on baseline body weight to estimate body mass index (BMI) at the time of HCC diagnosis could have led to patient misclassification because most HCC is associated with ascites, which can affect the BMI calculation and definition of obesity. Results from an ongoing case–control study indicated
means of BMIs at different age periods prior to HCC development were significantly larger for HCC patients as compared to healthy controls (Hassan, unpublished data) (Fig. 1.3).

**Thyroid Diseases**

Thyroid hormones play an essential role in lipid mobilization, lipid degradation, and fatty acid oxidation [92]. Patients with hypothyroidism may experience 15–30% weight gain [93] and insulin resistance [94, 95], which are significant factors of NASH. A recent study [96] reported that the prevalence of hypothyroidism in patients with NASH was significantly higher than in controls (15% vs 7.2%, respectively; $p = 0.001$). Such findings were later supported by Reddy and colleagues [97] from Mayo Clinic who assessed the association between hypothyroidism and HCC among 54 HCC patients of unknown etiology and 116 HCC patients related to HCV and alcohol. The study reported OR of 6.8 (95% CI, 1.1–42.1) for HCC development after adjusting for several confounding factors. Our recently published case–control study reported positive association between hypothyroidism and HCC among women [98].

Whether and why hypothyroidism causes HCC is not clear. However, the association between hypothyroidism and NASH can be explained by the underlying hyperlipidemia, decreased fatty acid oxidation, insulin resistance, and lipid peroxidation in patients with hypothyroidism. All of these conditions may enhance the susceptibility to chronic inflammation, DNA damage, and HCC development. Moreover, concurrent thyroid dysfunction among diabetic patients may exacerbate the coexisting diabetes-induced dyslipidemia and may explain our observation of HCC risk modification among patients with hypothyroidism and diabetes [98].
Obesity and hyperinsulinemia may increase the level of insulin-like growth factor-1, which in turn may reduce hepatic synthesis and blood concentration of sex hormone-binding globulin (SHBG) [99, 100], a glycoprotein produced in the liver with high-binding affinity for testosterone and lower affinity for estradiol. Independent of obesity, there is sufficient evidence that thyroid hormones have a positive effect on hepatic SHBG synthesis and that patients with hypothyroidism may experience a lower level of SHBG [101]. Thus, a decreased level of SHBG may lead to increased plasma testosterone and estradiol, both of which may promote cellular proliferation and inhibit apoptosis. Elevated levels of serum testosterone and testosterone to estradiol ratio have been proposed to be predictive of HCC development in Japanese men with cirrhosis [102]. Nevertheless, the fact that the association between hypothyroidism and HCC continued to be significant after adjustment for prior history of obesity suggested that other mechanisms of hepatocarcinogenesis were involved, especially among women.

**Cholelithiasis (Gallbladder Stones)**

The prevalence of gallstones in patients with cirrhosis is significantly higher than in the general population [103, 104]. This is partially attributed to the metabolic changes such as increased unconjugated bilirubin in bile secondary to hypersplenism, decreased cholesterol secretion, and decreased in apolipoprotein (apo) A-1 and AoA-II sections [105, 106]. A recent study reported significant association between gallbladder stones and HCC; the estimated OR (95% CI) was 14.75 (13.14–16.56) [107]. Nevertheless, the association between gallstones and HCC is difficult to assess from epidemiological studies due to recall bias among HCC patients and due to the subsequent cholecystectomy procedure with liver resection in patients with HCC. Therefore, it is not clear whether cholelithiasis is a risk factor for HCC or a consequence of the underlying chronic liver diseases in patients with HCC.

**Dietary Factors**

Most of the epidemiological evidence on diet and liver cancer is based on case-control studies and retrospective analysis. This type of assessment is subjective to recall bias due to the fact that patients with chronic liver diseases or cirrhosis may change their diet after being diagnosed with liver diseases. An example of the association between diet and HCC is HCC risk reduction (25–75%) among coffee drinkers who consume two to four cups of coffee per day as compared to non-coffee drinkers [108–110]. HCC risk reduction was also observed for the intake of eggs, milk, yogurt, vegetables, white meat, and fruits [111]. Moreover, the intake of dietary antioxidants, especially selenium and retinoic acid, showed a protective effect for HCC development in HBV carriers and cigarette smokers [112].
**Genetic Risk Factors**

**Familial Aggregation**

Familial aggregation of liver cancer has been reported. However, most of these studies were conducted among Asians, particularly in China [113–117]. Given the high prevalence of chronic infection with HBV and that vertical transmission of HBV is the major source for viral transmission among Asians, the reported association between a family history of liver cancer and HCC could be explained by clustering of HBV infection among members of the same family [118]. To avoid this obstacle, Yu et al. [117] matched 553 patients with HCC and 4,684 controls according to HBV infection status. They reported an OR of 2.4 (95% CI, 1.5–3.9) for HCC development in subjects with HBV and a family history of HCC as compared to subjects with HBV but no family history of HCC. A later study by the same investigators showed that familial segregation of HCC in HBsAg carriers is associated with familial clustering of liver cirrhosis [119].

A segregation analysis of Chinese HCC patients suggested that a Mendelian autosomal recessive major gene might also play role in HCC etiology [114]. In addition, first-degree family history of liver cancer in American and European populations is likely to be associated with HCC development independent of chronic infection with HBV and HCV [120]. Synergism between HBV/HCV and a family history of liver cancer was also noted by Hassan et al. [120] among Italian and American individuals.

**Inherited Diseases**

**Hereditary Hemochromatosis**

Hereditary hemochromatosis (HHC) is an autosomal recessive genetic disorder of iron metabolism that causes excessive intestinal absorption of dietary iron and deposition of iron in organs including the liver [121]. Recently, a major histocompatibility complex class I gene named \( HLA-H \) or \( HFE \) was cloned. Two mutations were described: Cys282Tyr (\( C282Y \)) and His63Asp (\( H63D \)) [122]. The \( C282Y \) mutation is more frequent in HHC [123]. There is growing evidence that even mildly increased amounts of iron in the liver can be damaging, especially when combined with other hepatotoxic factors such as alcohol consumption and chronic viral hepatitis. Iron enhances the pathogenicity of microorganisms, adversely affects the function of macrophages and lymphocytes, and enhances fibrogenic pathways [124, 125], all of which may increase hepatic injury caused by iron alone or by iron and other factors such as chronic HCV infection.

Indeed, a synergistic relationship between HCV and iron overload from hemochromatosis has been suggested [126]. In a study by Hayashi et al., iron depletion improved liver function tests in HCV-infected individuals [127]. In a study by Mazzella and colleague response of chronic HCV to interferon was shown to be related to hepatic iron concentration [128].
Possible factors contributing to the actions of iron in chronic viral hepatitis include enhancement of oxidative stress and lipid peroxidation, exacerbation of immune-mediated tissue inflammation, enhancement of the rate of viral replication, enhancement of the rate of viral mutation, possible impairment of cellular immunity or humoral immunity, and possible impairment of T-lymphocyte proliferation and maturation [129].

**α₁ Antitrypsin Deficiency**

α₁ antitrypsin deficiency (AATD) is an autosomal dominant genetic disorder characterized by a deficiency in a major serum protease inhibitor (Pi) [130]. AATD is caused by a mutation in the 12.2 kb α₁ antitrypsin gene on chromosome 14 [130]. Over 75 different Pi alleles have been identified, most of which not associated with disease [131]. A relationship exists between Pi phenotypes and serum concentrations of α₁ antitrypsin. Thus, the MM phenotype (normal) is associated with a serum concentration of 100%, MZ 60%, SS 60%, FZ 60%, M 50%, PS 40%, SZ 42.5%, ZZ 15%, and Z 0 to 10%. The most common deficiency variant, PiZ, in its homozygote state is often associated with liver cirrhosis and liver cancer [132]. The role of the heterozygous PiZ state in the development of primary liver cancer is controversial [133–135]. However, there is increasing evidence suggesting that chronic liver disease develops only when another factor such as HCV infection is present and acts as a promoter for the liver damage process. α₁ antitrypsin is an acute-phase reactant whose major role is to inhibit the actions of neutrophil elastase, proteases, and cathepsin G [136]. Any condition triggering the acute-phase response would be expected to stimulate the production of α₁ antitrypsin by the liver.

Therefore, it is suggested that chronic HCV infection could constantly stimulate the hepatocytes to produce the mutant α₁ antitrypsin, leading to more liver damage [137]. Other less frequent inherited disorders such as glycogen storage disorder disease type I (von Gierke’s disease) [138], Porphyria Cutanea Tarda [139], and Wilson’s disease [140] have been found to be complicated to HCC. However, the interactions between these diseases and other established risk factors such as HCV or HBV have not been studied.

**References**

1 Epidemiology and Pathogenesis of Hepatocellular Carcinoma