Underlying Liver Disease

4

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4.1 Introduction

Hepatocellular carcinoma (HCC) is an increasing form of cancer: it is estimated that, by 2025, more than one million individuals will be affected by HCC annually [1]. HCC represents the sixth most common cancer worldwide and the third most common cause of cancer-related mortality. HCC typically develops on a background of chronic liver disease or cirrhosis in 70–90% of all cases, but about 20% of cases can develop in the non-cirrhotic liver [2]. All risk factors for liver cirrhosis play a role in hepatocellular carcinogenesis, and liver cirrhosis "per se" is a precancerous condition. In patients affected by HCC, chronic liver disease or cirrhosis due to hepatitis C virus (HCV), hepatitis B virus (HBV), hemochromatosis, non-alcoholic steatohepatitis (NASH), alcoholic hepatitis, autoimmune hepatitis, primary sclerosing cholangitis, and primary biliary cholangitis are the most common underlying diseases that predispose to the development of HCC. Certain drugs and toxins are also risk factors for HCC. Furthermore rare monogenic syndromes, such as alpha 1-antitrypsin deficiency, glycogen storage disease type I, hemochromatosis, acute intermittent and porphyria cutanea tarda, as well as hereditary tyrosinemia type I are associated with a high risk of HCC.

There is geographic heterogeneity in the etiologic factors for HCC, which vary across countries worldwide. HCV infection and alcoholic liver disease are the main cause of liver cancer in developed countries and the predominant causative factors in Western Europe, whereas HBV infection is the primary risk factor in most developing countries and particularly in most parts of Asia, South America and Africa. HCC related to NASH is increasing worldwide. Some studies report different prevalence rates of NASH as the underlying cause of HCC, ranging between 4% and 22% in developed countries, as reported by Michelotti et al. [3]. In another population-based

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study, in the United States, non-alcoholic fatty liver disease (NAFLD) accounted for 59% of HCC cases, with a cumulative incidence rate of 0.3% over a 6-year follow-up [4]. NAFLD/NASH is an increasing cause of HCC in developed countries.

4.2 Viruses and Hepatocellular Carcinoma

Chronic HBV, HCV and hepatitis delta virus (HDV) infections are the traditional viral risk factors associated with the development of HCC. HCV-related HCC pathogenesis is thought to occur indirectly via chronic inflammation and oxidative stress with subsequent cirrhosis, while HBV can have also a direct oncogenic mechanism, sometimes independent from the development of liver cirrhosis.

4.2.1 Hepatitis B Virus

HBV infection is the cause of 60% of HCC cases in Africa and East Asia while it is the underlying cause of HCC in about 20% of cases in Western Europe. HBV can integrate into the host cell genome causing insertional mutagenesis and leading to the activation of oncogenes. For this reason HBV is associated with an increase risk of developing HCC even in the absence of liver cirrhosis.

HBV is a double-stranded circular DNA virus. The incorporation of the virus into the human gene causes inactivation of protein p53, a transcription factor that suppresses tumor growth. The HBx protein of HBV can bind to p53 forming a protein complex and inactivating many functions of p53, including apoptosis. A p53 mutation is present in 30–60% of HCC patients. Furthermore, HBx sequesters p53 in the cytoplasm and prevents it from entering the nucleus. Inactivation of p53 is one of the factors implicated in oncogenesis of HBV-related HCC. An abnormal activation of the B catenin signaling pathway has been observed in more than 60% of patients with HCC [5]. The activation of this pathway is related to the occurrence of the stemness and drug resistance of HCC cells [6].

Levels of reactive oxygen species (ROS) are increased in the blood and liver of patients with hepatitis B. ROS has an important role, as demonstrated in several studies, in promoting HBV-related liver fibrosis and cancer [7]. The risk of HCC among patients with HBV infection is approximately 2–5% and the disease can develop even in the absence of liver cirrhosis. The risk of developing HCC is reduced by approximately 50–60% in patients treated with antiviral therapy with virological response (VR) [8]. But VR seems not to significantly reduce the overall incidence of HCC when a patient has already progressed to liver cirrhosis [9].

4.2.2 Hepatitis C Virus

Chronic HCV is the most common underlying disease in Europe, North America and Japan. HCV is an RNA virus that does not integrate in host cell genome and for this reason the risk of developing HCC is more common in patients with liver

cirrhosis. The risk of developing HCC is lower but persistent in cirrhotic patients who have reached a sustained virological response (SVR) after therapy with direct-acting antiviral (DAA). The relationship between HCV infection and HCC has been widely studied. HCV infection causes inflammation and necrosis of hepatocytes. Cell turnover due to inflammation induces, through poorly differentiated hepatocytes, dysplastic foci and lastly HCC [10]. HCV infection leads to endoplasmic reticulum (ER) stress and seems to alter calcium homeostasis, inducing oxidative stress. Some studies showed that excessive ER stress due to HCV replication, degrades p53 in the lysosomes so HCV infection disrupts p53 function through activation of a protein kinase [11]. Reduction of p53 tumor suppression can favor development of HCC. High intracellular ROS levels, as in HBV infection, seem to promote hepatocarcinogenesis [12].

HCV infection and in particular HCV core and NS5 proteins can activate telomerase reverse transcriptase (TERT) expression and reverse transcriptase activity. The increased TERT activity has been found to be associated with HCC [13].

HCV can also activate the Wnt/beta-catenin and subsequent activation of pro-survival genes, a pathway that has been shown to promote HCC.

In conclusion, HCV infection and in particular liver cirrhosis due to HCV can predispose indirectly to the development of HCC, but other direct mechanisms of HCV-related HCC oncogenesis exist and can add to the risk for HCC development.

4.2.3 Hepatitis Delta Virus

HDV has not yet been included in the list of carcinogenic viruses, but evidence suggests that the risk of developing HCC is higher in patients with chronic hepatitis D compared to those infected with HBV. HDV replicates in the nucleus of hepatocytes and interacts with several cellular proteins, modulating their expression.

HDV may alter multiple cellular signaling pathways involved in inflammation, oxidative stress, apoptosis, and cellular proliferation. HCC associated with HDV was shown to be characterized by the upregulation of genes involved in the control of DNA replication, and DNA damage and repair. Genome instability due to HDV infection is an important mechanism of hepatocarcinogenesis [14]. This genomic profile is peculiar to HDV and distinct from that of HBV-associated HCC, suggesting that these two viruses promote hepatocarcinogenesis by different mechanisms.

4.3 Non-alcoholic Fatty Liver Disease and Non-alcoholic Steatohepatitis

Over the last decade, NAFLD has become an increasing cause of HCC. NAFLD is considered as the hepatic manifestation of the metabolic syndrome and is closely associated with obesity and diabetes. NAFLD is not always associated with an evolution towards liver cirrhosis, but at least 20–30% of patients develop liver disease with necroinflammation and fibrosis. This condition is known as non-alcoholic steatohepatitis (NASH). Patients with NASH have an increased risk of developing HCC.

NAFLD is characterized by excessive lipid accumulation (steatosis) with evolution in some cases into NASH. Liver cirrhosis and HCC are complications of NASH when this condition is not properly treated. It is interesting to note that HCC is reported also in non-cirrhotic NASH patients [15].

A meta-analysis has demonstrated that patients with diabetes mellitus have a higher risk of developing HCC compared to non-diabetic patients [16]. Overweight patients in a similar way have an increased risk for HCC. NAFLD/NASH, as reported before, are the expression of a metabolic syndrome characterized by diabetes mellitus, insulin resistance, obesity and hypertriglyceridemia.

HCC associated with NAFLD/NASH could have different mechanisms. Hepatic lipid accumulation progresses to necroinflammation leading to hepatocarcinogenesis as a consequence of different conditions such as insulin resistance, hyperinsulinemia, dyslipidemia, oxidative/endoplasmic reticulum (ER) stress, genetic predisposition, dysbiosis in the gut microbiome and altered response of the immune system. Insulin resistance leads to an increase in intracellular free fatty acids (FFA). Elevated FFA β oxidation induces oxidative stress and the release of ROS and of various inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and leptin. There is a link between oxidative/ER stress and progression to HCC. Oxidative stress promotes carcinogenesis by activation of JNK kinase and inactivation of the p53 tumor suppressor gene (*TP53*) [17]. Iron overload is frequently observed in NASH patients and is related to insulin resistance. Intracellular iron overload due to increased production of hepcidin can induce DNA damage that may predispose to HCC [18]. Other studies have also identified the role of the immune system and, in particular of $CD8^+$, $CD4^+$ and Kupfer cells and of altered intestinal gut microbiome in hepatocarcinogenesis in patients with NASH/cirrhosis.

4.4 Alcoholic Fatty Disease

The prevalence of alcoholic fatty liver disease (AFLD) is increasing throughout the world. About 26–30% of HCC can be attributed to alcohol. Central and Eastern Europe and tropical Latin America have a higher incidence of drinkers. There is some evidence that females are more susceptible to the toxic effects of alcohol than males. It was demonstrated that women have lower levels of gastric alcohol dehydrogenase activity and for this reason they are more susceptible to the hepatotoxic effects of alcohol. Furthermore, some studies have demonstrated that Whites have lower ethanol metabolizing enzymes in the liver, compared to Blacks and Hispanics [19]. Progression to cirrhosis and mortality is higher in AFLD (36%) compared to NAFLD (7%). AFLD has a similar mechanism of liver damage compared to NAFLD. Alcohol is metabolized into acetaldehyde by alcohol dehydrogenase (ADH), the CYP2E1 enzyme represents the major pathway involved in the metabolism of ethanol. High cell concentrations of acetaldehyde and ROS are formed in the cell. Acetaldehyde is a potent carcinogen driving the tumorigenesis by the alteration of DNA while concomitant high concentrations of ROS can activate JNK kinase with subsequent induction of carcinogenesis [20].

Ethanol is also involved in "*de novo* cellular lipogenesis" with following steatosis and excess of intracellular FFA. Excess FFA, as observed in NAFLD, determines oxidative/ER stress, increase intracellular ROS levels with progression to HCC [20].

In conclusion AFLD induces cirrhosis and promotes HCC through a similar mechanism to NAFLD.

4.5 Hereditary Hemochromatosis

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism, with elevated iron deposition in most organs including the liver, leading to progressive liver dysfunction and cirrhosis. HCC is a complication of HH nearly always occurring in liver cirrhosis. About 80–85% of individuals with HH are *C282Y* homozygotes and are at risk of developing liver cirrhosis. Other mutations in the high iron gene are *C282Y*/*H63D* compound heterozygosis. Excessive iron in the liver may act both directly and indirectly to induce carcinogenesis. Free intracellular iron, which is present when iron binding capacities of the plasma transferrin or intracellular ferritin are surpassed, interacts with H_2O_2 with formation of Fe^{3+}. Superoxide anions can reduce Fe^{3+} back to Fe^{2+}. Increased accumulation of Fe^{2+} in the cytosol enhances generation of ROS, whose toxic effects on proteins and DNA promote carcinogenesis. HH patients have higher rates of *TP53* gene mutations and decreased p53 protein activity in the liver, thus facilitating hepatocarcinogenesis [21]. Increased intracellular iron is also present in chronic liver diseases such as AFLD, NAFLD, and viral infections, contributing to the pathogenesis of HCC in other liver diseases.

4.6 Autoimmune Hepatitis and Primary Biliary Cholangitis

In autoimmune hepatitis (AIH), chronic liver inflammation and interface hepatitis are present which cause liver inflammation and fibrosis. While early diagnosis and treatment avoid progression to cirrhosis, the persistence of damage leads to liver fibrosis. A major risk factor for HCC in AIH is cirrhosis, and cirrhosis appears as "a sine qua non" condition for the development of HCC in AIH patients. A similar risk for hepatobiliary cancer is present in primary biliary cholangitis (PBC). The incidence of HCC in AIH is 3.06 per 1000 person-years while it is 4.1 per 1000 person-years for PBC. These data support the importance of regular monitoring of disease severity in AIH and PBC, with initiation of HCC screening in patients who progress to cirrhosis [22].

4.7 Wilson Disease

Wilson disease is caused by accumulation of copper in the liver, brain or other organs due to mutation of *ATP7B* gene that encodes a protein that helps in excretion of copper in the bile canaliculus. This results in toxic levels of copper in the

hepatocytes. Hepatocyte apoptosis and mitochondrial oxidative injuries is the mechanism of copper injury in hepatocytes. Due to the availability of chelating agents, life expectancy of these patients has now increased. In some studies the risk of HCC was low even in cirrhotic patients and this leads the authors to state that regular surveillance for HCC is not required. It has been postulated that a high hepatic copper level is protective against hepatic oncogenesis. Based on animal studies, it has been suggested that excessive copper accumulation might have a protective effect on hepatocarcinogenesis. On the other hand, in the Long-Evans Cinnamon rat model for Wilson disease, persistent copper accumulation resulted in an increased risk of HCC which could be prevented by administration of D-penicillamine [23]. Therefore carcinogenesis is thought to be the result of liver injury leading to chronic inflammation and cirrhosis due to chronic copper accumulation [23].

4.8 Alpha 1-Antitrypsin Deficiency

Alpha 1-antitrypsin (A1AT) is the most abundant liver-derived glycoprotein in plasma. Hereditary deficiency of A1AT in plasma leads to an accumulation of polymers of A1AT mutants in the ER of hepatocytes. One of the clinical manifestations of A1AT deficiency is liver disease in childhood and cirrhosis and/or HCC in adulthood. Mutations of A1AT results in two pathologic genotypes called PiZZ and PiSZ. The PiZZ A1AT genotype is associated with liver damage and high risk for HCC. Accumulation of A1AT variants in ER may potentially induce multiple signaling events related to ER stress. ER stress induces an altered regulation of several genes driving proliferation and tumorigenesis; furthermore there is a secondary activated mitochondrial autophagy. The final results are liver inflammation and carcinogenesis [24].

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