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REVIEW ARTICLE

# Non-Viral Causes of Hepatocellular Carcinoma

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**Abstract**
*Introduction* Hepatocellular carcinoma (HCC) is a major cause of cancer worldwide. The vast majority of cases occur in individuals with a chronic HBV or HCV infection. In addition, a number of metabolic diseases of the liver are associated with the development of HCC.
*Pathophysiologic Mechanisms* The mechanisms responsible for the progression of the metabolic liver disease and HCC differ from those associated with viral liver disease.
*Conclusions* The purpose of this report is to describe the mechanisms responsible for the disease progression and HCC in case of metabolic liver disease. A secondary goal is to identify the frequency of HCC development in the disorders described.

**Keywords** Hepatocellular carcinoma · Cancer · NAFLD · NASH

## Introduction

Hepatocellular carcinoma [1–6] is a major cause of cancer and cancer-related deaths worldwide. The vast majority of cases are a consequence of a preexisting chronic viral infection due to either hepatitis B with or without associated hepatitis D or hepatitis C [7–9].

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The number of cases directly related to hepatitis B (HBV) infection has remained stable worldwide with most of the cases of HBV-associated HCC occurring in Southeast Asia and Sub-Saharan Africa [7–9].

In contrast, the number of cases of HCV has increased and is expected to steadily increase over the next 20–30 years as a result of the continuing problem of HCV infection and disease chronicity [4, 10–13].

The majority of cases of HCV-related HCC occur in Europe and the Americas. The number of HCC cases that occur, independent of a preexisting viral infection, is increasing worldwide as a consequence of the global increase in individuals manifesting one or more of the components of the metabolic syndrome that include obesity, coronary artery disease, hyperlipidemia, type 2 diabetes mellitus, gout, sleep apnea, and nonalcoholic fatty liver diseases (NAFLD) or nonalcoholic steatohepatitis (NASH) [14–23].

In addition, a much smaller yet substantial number of cases are a consequence of chronic alcohol-associated cirrhosis or one or a large number of inherited metabolic liver diseases, the most common of which are alpha-1 antitrysin deficiency, hemochromatosis, Wilson's disease, and type 1 tyrosenemia [24].

Finally, the few residual cases of non-viral HCC that have been ascribed to environmental exposures to include aflatoxin in contaminated grains, tobacco use, oral contraceptives, and use of anabolic steroids.

## Pathophysiologic Mechanisms

The underlying mechanisms responsible for these non-viral-associated HCC are in general a consequence of an epigenetic event that persists and disrupts the normal cell cycle that

contract cellular proliferation, differentiation, and senescence or a genetic polymorphism that enhances the risk for HCC development [24].

Considerable data exist for the former epigenetic factor hypothesis while relatively little and variable data exist for the presence of an intrinsic genetic mutation leading to the development of HCC other than those associated with well-recognized metabolic liver diseases.

Regardless of the specific epigenetic mechanisms involved, enhanced oncogene transcription or its promotion, reduced degradation of a cyclin, DNA, RNA on regulatory protein occurring as a result of hyper- or hypo-methylation of DNA and/or RNA, free radical induced per oxidation or the presence of either reactive oxygen or nitrosyl compounds, occurring as a result of oxidative stress.

The vast majority of non-viral-associated HCC manifest biochemical evidence of insulin resistance and/or deregulation of a growth factor (including insulin) [25, 26].

As a direct consequence of these various mechanisms leading to the development in HCC, it is not surprising that HCCs are heterogeneous in their growth rates, degree of cellular differentiation (morphology), cellular origin, and potential for metastasis.

## Representative Disease Examples

### Alcoholic Liver Disease

It is estimated that 15–20% of alcoholics with cirrhosis develop HCC at a rate of 3–4%/year. In rare cases, occurring in the absence of cirrhosis either an unrecognized low-grade chronic hepatitis C or an occult case of HBV infection can be identified and manifested by H B core antibody positivity.

The principal pathophysiologic mechanism leading to HCC in chronic alcoholics, however, is an oxidative stress induced within the liver as a direct consequence of the metabolism of ethanol, its first metabolic product acetaldehyde, and possibly acetate by mitochondria and the rich endoplasmic recticulum found in the hepatic cytosil [27, 28].

The resultant loss of ATP production and cellular injury occurring as a result of membrane phospholipid and protein oxidation, protein carbonyl formation, and the production of 1-hydroxyethanol radicals as well as other alkyl free radicals leads to altered cell signaling mechanisms, transcription, and translation errors that ultimately result in the development of HCC.

The consequences of ethanol related nutritional perturbations that include folate deficiency, pyridoxylphosphate deficiency, and a diet rich in carbohydrates and lipids at the expense of protein are additional factors that contribute to the enhanced expression of protocongenes and oncogenes and the risk of HCC development [29–35].

### NAFLD and NASH

The current worldwide epidemic of NAFLD occurring in children as well as adults and in lean as well as obese individuals has resulted in a remarkable increase in the number of cases of HCC occurring in the Western World and more opulent numbers of the Third World who have no evidence for either current or past HBV and/or HCV infection [16–20].

Currently, NAFLD is considered a benign condition that can progress to NASH in approximately 20% of cases. In turn, 20% of NASH cases are thought to progress to cirrhosis. HCC can develop in individuals with NASH combined with cirrhosis and also albeit less often in cases with NASH occurring in the absence of cirrhosis. The actual rate of HCC development in NASH-associated cirrhosis is estimated to be similar to that occurring in alcoholics with cirrhosis (3–4%/year). There are no current estimates of the rate of HCC development in cases of NASH without cirrhosis but increasingly, such cases are being recognized.

The pathophysiologic mechanisms leading to HCC in NASH and NASH plus cirrhosis appear to mirror the events occurring in alcoholic liver disease enhanced further by the presence of insulin resistance.

Insulin resistance is known to be associated with a reduction of tyrosine, phosphorytation in many different cells and organs. As a result, alternate cellular pathways affecting cell proliferation and differentiation are upregulated. The combination of the upregulation of proteins responsible for cellular proliferation and differentiation coupled with the net oxidation stress induced by the hepatic metabolism of triglycerides is a potent driving force for the development of HCC.

### Hepatic Disorders of Iron and Copper Metabolism

Cases of both HFE and non-HFE associated determined hepatic iron overload (hemochromatosis) as well as cases of excessive hepatic copper retention as occurs in Wilson's disease result in the development of cirrhosis, and in 7.5–30% of these cirrhotic cases, a HCC develops. Rare cases of non-cirrhotic HCC have been reported in both forms of hepatic metal overload [36–42].

The pathophysiology of these disorders is characterized by mitochondrial dysfunction with the production of reactive oxygen and reactive nitrozyl species that characterize oxidative stress. The resultant disruption of cell cycling in these two disorders is characterized by enhanced cellular replication as well as an increased apoptosis rate.

Insulin resistance associated with cirrhosis is common in individuals with either hepatic iron or copper overload and may further enhance the risk of HCC occurring as a consequence of the more obvious oxidation stress present in these two disorders. Iron reduction therapy reduces the prevalence of cirrhosis and, as a result, HCC in cases of hemochromatosis. Most cases of Wilson's disease are recognized clinically only after cirrhosis has developed, but it is likely that copper reduction therapy in precirrhotic individuals with Wilson's disease identified by means of family screening may lead to a reduced rate of HCC development as well.

### Alpha-1 Antitrypsin Deficiency

Alpha-1 antitrypsin deficiency is an autosomal recessive disorder resulting in the expression of a defective alpha-1 antitrypsin protein as a consequence of the presence of an abnormal allele. The abnormal alleles currently recognized in cases of alpha antitrypsin deficiency include, Z, S, F, and null alleles. Individuals who are homozygous for an abnormal allele or are a compound heterozysate having two different abnormal alleles manifest the clinical disorder.

HCC is common in children with alpha-1 antitrypsin deficiency and cirrhosis as well as in adults who are 50–60 years of age. In the adult cases of alpha-1 antitrypsin with cirrhosis, HCC is reported to occur in 31–67% of cases [43–45].

The hepatic endoplasmic reticulum and mitochondrion in individuals with alpha-1 antitrypsin deficiency demonstrate morphologic and biochemical abnormalities. As a result, the sum of the many different cellular injuries associated with oxidative stress associated with mitochondrial injury is thought to be the driving force for HCC development in cases of alpha-1 antitrypsin deficiency.

### Tyrosinemia Type 1 or Hepatorenal Tyrosenemia

Tyrosenemia type 1 is an autosomal recessive disorder due to deficiency of Fummaryl acetoacetate hydrolase which results in an accumulation of Fummaryl acetonatate and malyl aceto acetate [46].

Type 1 tyrosinemia has a worldwide prevalence of 1/100,000 live births but occurs at an increased rate as high as ½,000 live births in specific geographic locations [47]. Apoptosis characterizes the histological appearance of the liver affected by type 1 tyrosinemia [48]. The apoptosis signal appears to be fumaryl acetoacetate. Both fumarylacetoacetate and malyl acetoacetate are alkylating agents that induce DNA and RNA mutagenesis. As is the case in all of all of the preceding diseases, a prominent oxidative stress occurs in tyrosinemia as a consequence of the metabolism of malylacetone, fummarylacetone, succinylacetate, and succinylacetone.

## Summary and Conclusions

The epigenetic and resultant genetic alterations produced as a consequence of an excessive oxidative stress characterizes the diseases of metabolism that are associated with the development cirrhosis and ultimately HCC.

These metabolic alterations result in gene over- and underexpression enhanced proliferation, reduced apoptosis, proto-oncogene, oncogene over expression, and reduced levels of cycling dependent kinase inhibitors.

The specific alteration that is most evident in a given disease associated with HCC development varies with and across diseases making a more specific description of the pathogenesis of HCC any difficult.



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