9 Metabolic Disease and Hepatocellular Carcinoma

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ABSTRACT

The pathophysiologic mechanisms recognized as inducing changes in the cell cycle and its regulation that enables carcinogenesis to occur are presented. More importantly, the mechanisms thought to be most important in each metabolic disorder that can terminate in the development of a hepatic cancer are identified. Often more than one mechanism is involved and it is the sum mutation of these metabolic events with environmental hazards and exposures that enable a cancer to develop or not in an individual with a metabolic disease having an association with hepatic cancer.

Key Words: Free-radical injury; oxidative stress; epigenetic dysfunction; inducible genetic errors; hypo/hypermethylation; cell cycle disruption; genetic disorders

1. INTRODUCTION

Hepatocellular carcinoma is the most common cause of primary liver cancer accounting for more than 80% of cases *(1, 2)*. It is only second in frequency to all forms of metastatic cancers to the liver combined (colon, stomach, pancreas, breast, lung) as a cause of liver cancer. More than 1 million deaths each year occur as a result of hepatocellular carcinoma, and it accounts for one-third of all the cancer-related deaths occurring annually worldwide *(3, 4)*. The ratio of hepatocellular cancer deaths occurring annually to the incidence of new hepatocellular carcinomas in the population ranges between 0.85 to 0.90 and documents the severity of the disease process once identified *(5, 6)*.

The risk factors for hepatocellular carcinoma vary geographically and include cirrhosis of any cause, chronic hepatitis (especially HBV), toxin-induced liver diseases (alcohol, tobacco, aflatoxins, other chemicals and drugs), chronic viral hepatitis (HBV and HCV), and various metabolic liver diseases *(7)*. This last group is rather small but it is an important group to recognize and, as a result, to screen for the development of hepatocellular carcinoma. If identified early, liver transplantation cures not only the hepatic

cancer but also the metabolic abnormality and the cirrhosis present in these cases.

Other risk factors for hepatocellular carcinoma include male gender, increasing age at the time of HBV or HCV infection, obesity, diabetes mellitus, nonalcoholic fatty liver disease, especially nonalcoholic steatohepatitis and chronic cholestasis. Each of these factors can coexist in an individual with a metabolic liver disease and affect the disease outcome and potentially enhance the risk for hepatic cancer. Despite the impressive evidence for the prevention and control of HBV infection occurring as a consequence of childhood vaccination programs and current antiviral therapies, the incidence of hepatocellular, at least in the western world, is increasing rather than decreasing *(2–4)*. This increase in hepatocellular carcinoma cancer is primarily due to the increase in cases associated with HCV infection, nonalcoholic steatohepatitis, cryptogenic cirrhosis, obesity, and diabetes mellitus, all of which except HCV are components of the metabolic syndrome. It is interesting to consider the potential role of being heterozygous for genes associated with genetic hemochromatosis, alpha 1 antitrypsin deficiency, methylenetetrahydrofolate reduction deficiency, and other genetic disease in rare cases with a newly recognized hepatocellular carcinoma. The vast majority of such cases manifest biochemical evidence of insulin resistance which is characterized by an increased insulin level relative to the plasma glucose level or by an increased glucose level together with normal or even increased serum insulin levels. It may well be that insulin resistance per se may be the underlying factor responsible for the development of hepatocellular carcinoma in most of these cases of hepatocellular cancer. Certainly, growth factors including insulin are recognized as playing at least some role in the pathogenic mechanisms culminating in the development of hepatocellular carcinoma *(8)*. On the other hand, increased serum insulin levels can be the result of the metabolic changes taking place with in the liver.

Hepatocellular carcinomas are heterogeneous in their morphology, growth rates, and potential for metastasis. The possible precursor(s) of the different phenotypes are still unknown. These differences may arise in part as a result of the many different cells from which a given hepatocellular carcinoma may occur. These include first mature (or dividing) hepatocytes, oval cells (periductular cells) (stem cells found adjacent to the ducts of Hering), and potentially stem cells of bone marrow origin present within the liver. Moreover, it is possible that in individuals with multifocal or asynchronous hepatocellular tumors each tumor may have a different cellular origin which can account for their different morphogenesis and biologic characteristics.

Essentially all hepatic cancers arise as a consequence of a chromosomal aberration that can arise during cellular proliferation, when cell damage and death have occurred. The specific disruption involved in any particular case or time can vary depending on the presence of one or more epigenetic or

genetic abnormalities that are present and disrupt the normal regeneration process.

Under normal conditions, the cell cycle is tightly regulated by various phosphorylating enzymes and is promoted by a variety of proteins termed cyclins which when combined with a phosphorylated kinase form a complete catalytic complex that controls cellular regeneration at various points in the cell cycle. Other proteins regulate programmed cell death (apoptosis) which limits cellular regeneration and proliferation.

Inflammation induces cellular injury on one hand and cytokine production and secretion that can result in an enhancement of cellular regeneration on the other hand. Moreover, normal control mechanisms that regulate the cell cycle *(9)* may be disturbed by repeated inflammatory flairs. Regardless of the specific etiology, hepatocellular carcinoma only develops when the control mechanisms regulating cell cycling and renewal or death are disrupted. These disruptions are multiple and include both epigenetic and genetic effects. The various epigenetic effects that can lead to an increased transcription of an oncogene or its promotion are either an increased transcription or a reduced degradation of a cyclin, DNA, RNA, or regulatory protein as a consequence of either hyper- or hypomethylation of DNA or RNA and free-radical injury (peroxidation) as a consequence of a reactive oxygen (ROS) or nitrosyl (RNS) species that occurs as a consequence of oxidative stress. Ultimately, epigenetic processes lead to genetic defects that result in cell cycle disruption.

The principal mechanism by which a nonviral metabolic liver disease progresses to cirrhosis and ultimately hepatocellular carcinoma is a result of oxidative stress induced as a result of cell injury, inflammation, followed by disturbed cellular regeneration and proliferation or reduced apoptosis.

2. OXIDATIVE STRESS

Reactive oxygen species (ROS) and reactive nitrosyl species (RNS) are unstable short-lived molecules generated by oxygen-utilizing cells. They are produced in either the mitochondria or the endoplasmic reticulum as a consequence of stress along an oxygen-utilizing metabolic pathway which contains an electron transport chain or as a result of metabolism involving either a cytochrome P450 enzyme system, xanthine oxidase, nitrous oxide synthesis, lipoxygenase, cyclooxygenase, or NADPH oxidase. Mitochondria because of their role in energy (ATP) production are a major source of ROS which are generated at two sites within the electron transfer (respiratory) chain within mitochondria: complex I (NADH/ubiquinone oxidoreductase) and complex III (ubiquinone/cytochrome oxidoreductase). Of these two sites, the more important is complex I where molecular oxygen (O2) is

converted to singlet oxygen (O^-) by the mitochondrial P450 cytochrome system in the liver, kidney, and to a lesser degree muscle resulting in the generation of ROS when stressed by either an excessive metabolic load (substrate requiring oxidation by mitochondria) or as a result of a reduced antioxidant (particularly glutathione) supply within mitochondria. When glutathione levels are inadequate, the catabolism of hydrogen peroxide (H_2O_2) within mitochondria is reduced as mitochondria do not contain catalase, the enzyme principally responsible for metabolizing H_2O_2. As a result, the unmetabolized H_2O_2 reacts with ferrous (Fe^{+2}) to produce the highly toxic hydroxyl (OH^-) radical. Singlet oxygen (O^-) can react with ROS and RNS activating cell-signaling pathways associated with kinase-linked receptors resulting in phosphorylation of growth-regulating pathways. They also oxidatively alter proteins, DNA, RNA, and lipids which can alter enzyme activity, alter both transcription and translation mechanisms, induce DNA strand breaks, and alter lipid structure and function. Each of these mechanisms disrupts normal cellular function. Moreover, each of these disruptions of critical cellular molecular mechanisms occurs not just in isolation in one cell but rather all together under conditions of oxidative stress amplifying the resultant cellular disruption that occurs.

The transition metals (iron and copper) which are abundant in liver cells accelerate the generation of ROS and RNS and activate the conversion of lipid peroxides into alkoxyl- and peroxyl-radicals which are highly reactive and have a longer half-life than the primary ROS and RNS. These same metals accumulate excessively in many liver disease conditions (hemochromatosis, Wilson's disease, alcoholic liver disease, nonalcoholic fatty liver disease, and nonalcoholic steatonecrosis, and any disease process associated with chronic cholestasis) and can contribute, at least in part, to the summation of events leading to the development of hepatocellular carcinoma in individuals with a metabolic liver disease as it happens when continuous toxic exposure takes place such as under continuous aflatoxin intake with the food.

3. ALCOHOLIC LIVER DISEASE AND HEPATOCELLULAR CARCINOMA

Alcoholic liver disease is composed of a spectrum of histological pathologies ranging from macrovesicular steatosis (fatty liver) to alcoholic hepatitis (fat, inflammation with polymorphonuclear leukocytes, a characteristic sinusoidal fibrosis, and the presence of Mallory bodies in ballooned hepatocytes) to alcoholic hepatitis plus cirrhosis and hepatocellular carcinoma occurring in cases with cirrhosis with or without alcoholic hepatitis *(10)*. Individuals with each of these histopathologic conditions can be either asymptomatic

or symptomatic. In general, the liver injury tests in alcoholic liver disease are characterized by an AST level greater than that of the ALT value. The alkaline phosphatase levels are highly variable depending on the severity of injury, presence of cirrhosis, and presence of bile duct injury/destruction. Hepatic cancer develops most frequently in those with cirrhosis with or without associated alcoholic hepatitis. Approximately 10–15% of alcoholics develop cirrhosis and HCC occurs in 15–20% of these cases at a rate of 3–4% per year. However, HCC can also develop in individuals consuming daily amounts of alcohol without apparent development of cirrhosis.

The role of chronic hepatitis C, and to a much lesser degree the presence of chronic hepatitis B (either evident or occult) in the pathogenesis of primary hepatic cancer in individuals with alcoholic liver disease, remains unclear but may well account for many of the cases of hepatocellular carcinoma in this population. This, however, does not negate the role of alcohol per se in initiating various metabolic changes that contribute to the pathogenesis of hepatic cancer in individuals with alcoholic liver disease. The pathogenetic mechanisms responsible for the development of primary hepatic cancer in cases of HBV and HCV are presented in other chapters and the reader is referred to those chapters for details. These mechanisms are likely to be additive and potentially synergistic to those due to alcohol abuse occurring in cases with alcoholic cirrhosis alone.

As a consequence of ethanol and acetaldehyde oxidation, an oxidative stress is induced in the liver which, if excessive and/or continuous as is the case in alcoholic individuals, results in mitochondrial and endoplasmic reticular injury, resulting in reduced ATP production and cell as well as organelle membrane disruption. These cellular and organelle changes occur in part as a consequence of membrane phospholipids and protein oxidation manifested as lipid peroxidation, protein carbonyl formation, the production of 1-hydroxyethanol radical, and other alkyl-free radicals *(11, 12)*.

Alcohol is not a carcinogen per se but acts as carcinogenic promoter as a consequence of the oxidative stress it induces and the downstream effects of the oxidative stress on cellular lipids, proteins, signaling pathways, DNA and RNA, and subsequent transcription and translation mechanisms. Alcohol-induced reductions in tissue folate levels enhance these effects by impairing transmethylation pathways *(13)*. A reduction in the level of cellular pyridoxaldol 5-phosphate induced by alcohol abuse is also important *(14, 15)*. Each of these effects results in enhanced DNA hypomethylation and upregulated gene expression particularly of proto-oncogenes and subsequently activated oncogenes *(16–19)*.

DNA methylation occurs predominantly at the fifth carbon atom of cytosine–guanine pairings *(20)*. This dinucleotide pairing frequently occurs within the promoter region of genes. Hypermethylation silences gene expression while hypomethylation which can occurs as a result of alcohol

abuse and its effect on folate, pyridoxine, and methionine metabolism is enhanced or unregulated gene expression. This enhanced gene expression and/or enhanced promoter activity enables enhanced binding of transcription factors to DNA and ultimately increased gene transcription *(21, 22)*.

Methionine adenosyltransferase (MAT) is the enzyme responsible for the synthesis of *S*-adenosyl methionine (SAMe). SAMe is the principal biological methyl donor and a precursor of aminopropyl groups utilized in polyamine synthesis and eventually DNA and RNA *(23)*. As such, it is an active participant in biochemical reactions essential for normal cellular proliferation. SAMe is also a precursor of glutathione, a major tissue antioxidant. MAT exists in two isoforms—MAT-1 and MAT-2 *(24, 25)*. MAT-1 is expressed primarily in the liver of adults while MAT-2 is expressed predominately in fetal liver. MAT-2 expression is enhanced in alcoholic liver disease and in human hepatoma and is associated with a reduction in MAT-1 *(26, 27)*. This enhanced MAT-2 expression is due to hypomethylation of the cytosine–guanine dinucleotide pair present in the MAT-2 promoter. This same promoter region has binding sites for heat-shock transcription factor, a STAT (signal transducer and activator of transcription), c-Myb, v-Myb, and GATA consensus binding sites, all of which enhance MAT-2 expression and upregulation of cellular proliferation *(28, 29)*. As a result of the different kinetic characteristics of MAT-1 and MAT-2, liver cells rich in MAT-2, have an overall greater MAT activity at physiologic concentrations of methionine and enhanced proliferative activity, critical factors in the progression from a dysplastic to a neoplastic cell and ultimately the pathway to hepatocarcinogenesis *(30, 31)*.

Each of these consequences of alcohol abuse (folate and B_6 deficiency, oxidative stress, MAT-2 induction, and many as-yet unrecognized adverse cellular events of alcohol abuse occurring in a cirrhotic) contributes to the pathogenesis of hepatocellular carcinoma in the alcoholic cirrhotic. As in the case of the synergism between alcohol and viruses, synergism can take place between alcohol and other substances contained in alcoholic beverages or alcohol and toxins like aflatoxin.

4. NONALCOHOLIC FATTY LIVER DISEASE (NAFLD), NONALCOHOLIC STEATONECROSIS (NASH), AND HEPTOCELLULAR CARCINOMA (HCC)

NASH was described by Ludwig and associates in 1980 *(32)*. In this initial report, the presence of obesity and type II diabetes mellitus as frequent comorbid conditions was recognized. Subsequently, the entire spectrum of NAFLD was recognized to include simple fatty liver, NASH, cirrhosis, and HCC. NAFLD per se is believed to be an innocuous health problem without sequelae, albeit an important and possibly the earliest clinical manifestation

of the metabolic syndrome. As a result of the increasing prevalence of obesity over the last two decades, NAFLD has become recognized as the most frequently recognized clinical hepatic disease in the western world being present in up to 20% of the adult population *(33)*. NAFLD can progress to NASH which is not an innocuous process but has the potential to progress to cirrhosis with NASH or cryptogenic cirrhosis, both of which can develop HCC without any residual histologic evidence of NAFLD. NASH is reported to be present in 3% of the adult population in the United States, a rate twice that of chronic hepatitis C (HCV) *(34)*. As a result, NAFLD and NASH are the two most common hepatic diseases occurring in adults in the United States and Western Europe. Most disturbing is the increased recognition of both NAFLD and NASH in children and adolescents *(33–36)*. Whether this increase in NAFLD and NASH in children will lead to an earlier age of onset of hepatoma in the adult population in the future remains to be determined. The development of NASH in adults is clearly associated with an increased risk of hepatocellular cancer *(37)*.

The metabolic syndrome is characterized by the presence of three or more of the following disease components: NAFLD, type II diabetes mellitus, hypertension, hyperlipidemia, especially hypertriglyceridemia, obesity, coronary artery disease, hyperuricacidemia, sleep apnea, and polycystic ovarian disease *(38)*. Typically, more than three of these disease processes exist in an individual with the metabolic syndrome. Obesity occurs in 30–100% of cases; type II diabetes mellitus occurs in 10–75% of cases; and hyperlipidemia in 2–50% in both adults and children with the syndrome. Coronary artery disease, hypertension, hyperuricacidemia, and polycystic ovarian disease can occur in children and adolescents with NAFLD/NASH but do so considerably less frequently than in adults. It should be noted that NAFLD and NASH can occur in lean individuals, with 3% of documented cases occurring in this population *(29)*. The obesity in individuals with the metabolic syndrome and NAFLD and/or NASH is typically truncal in character.

The recognition of the association between NASH and HCC appears to account in large measure for the observed increase in HCC rates in the United States particularly if cases with HCV disease and HCC are excluded from the calculation. Not only is NASH independently associated with HCC but it appears to enhance the risk of HCC development in cases of HCV-associated cirrhosis *(40)*. The rate at which HCC develops in NASH is not known but can be expected to parallel that seen in alcoholic liver diseases.

The pathophysiologic mechanisms that account for the development of NAFLD and its progression to NASH as well as the downstream complications of cirrhosis and HCC are not entirely clear but appear to be a consequence of a putative two hit processes *(41)*. The first hit is most likely an increase in hepatic fat as a consequence of hypertriglyceridemia. The oppo-

site may be the result of insulin resistance. Insulin resistance is known to lead to a diffuse reduction in tyrosine phosphorylation *(42, 23)* and a resultant disruption in cellular pathways affecting cell growth and differentiation. Triglycerides and fatty acids in the liver induce lipid peroxidation mechanisms as a result of an induction of P450 2E1 and 4A; a disruption of mitochondrial production of ATP; the induction, production, and secretion of inflammatory cytokines (IL-6, IL-8, TNF alpha); and enhanced lipopolysaccharide (LPS) hepato-toxicity *(44–47)*. Each of these events contributes to a state of considerable oxidative stress. As a result of the combination of lipid peroxidation, the production of reactive oxygen species (ROS), and reactive nitrosyl species (RNS), a reduction in hepatic and particularly mitochondrial antioxidants especially glutathione and ultimately a loss of mitochondrial energy production manifested by a loss of ATP production occurs. The latter event dramatically impairs endogenous attempts at cellular injury repair mechanisms. As a net result of this oxidative stress, both genetic and epigenetic mechanisms that contribute to carcinogenesis become manifest.

Importantly the risk of HCC in NASH-affected individuals appears to be limited to those with cirrhosis with or without concurrent NASH. As a result, screening for HCC is indicated only in those with cirrhosis. In such cases, the additional clinical findings of portal hypertension complicated by splenomegaly and thrombocytopenia (<75,000/μl) mandates surveillance for hepatic cancer and should be repeated at 6–12-month intervals utilizing hepatic ultrasound or triple-phase CT scanning procedures. In cases with either an iodine or an intravenous contrast allergy, an annual MRI with an iron-containing contrast agent can be substituted for the triple-phase CT scan.

5. HEMOCHROMATOSIS AND WILSON'S DISEASE AND HCC

Both iron and copper have the potential to be mutagenic as a result of oxidative stress *(48)*. An abundance of DNA adducts has been identified in the hepatic tissue of individuals with hemochromatosis and Wilson's disease *(49)*. DNA damage of hepatocytes exposed to iron has been demonstrated in vitro and most probably also occurs with copper exposure. Classic hemochromatosis is a common autosomal recessive disorder that occurs at a rate of 1/1,000 and is associated with the presence of abnormal alleles for HFE expression. These are C282Y, H63D, and S65C. The latter two alleles are very weakly associated with clinical iron storage and hepatic disease. The synergistic effects between increased hepatic iron storage and other toxins (e.g., alcohol) cannot be excluded as initiators of the hepatic cancerogenic process.

Other causes of "hemochromatosis" include juvenile hemochromatosis (a defect in hemojuvelin), transferrin receptor deficiency, and congenital atransferrinemia. Wilson's disease is also an autosomal recessive disorder that occurs at a rate of 1/30,000. It is due to a defective gene for a P-type ATPase. More than 100 different mutations for this disorder have been identified. The disease can present as an acute hemolytic process with fulminate liver failure, chronic hepatitis, cirrhosis with portal hypertension, or as a psychiatric/ neurologic disorder.

As noted in an earlier section of this chapter, mitochondrial production of ROS and RNS occurring as a consequence of oxidative stress represents a prime source of reactive species in the liver of individuals with either hemochromatosis or Wilson's disease. In both diseases, biochemical (functional) and histological disruption of mitochondria can be demonstrated and contribute to an increased rate of apoptosis, enhanced cellular replication, and a disruption in normal cell cycle functioning.

In Europeans with hemochromatosis, an increased frequency of the p53 tumor suppressor mutation has been reported and contributes to reduced hepatic DNA repair, further enhancing the development for a hepatic cancer *(50, 51)*.

Hepatocellular carcinoma is reported in 7.5–30% of cases of hemochromatosis *(32, 57, 58, 59, 60)*. Almost all the cases have been reported in cirrhotics but at least two cases have been reported in noncirrhotics (32 J). Age >55, the presence of concomitant diabetes mellitus, HbsAg, and alcohol abuse each increases the risk of cirrhosis and hepatocellular carcinoma in individuals with hemochromatosis. Iron reduction therapy was not been associated with a reduced risk of hepatocellular carcinoma in cirrhotics. Hepatocellular carcinoma was found to occur in cirrhotic livers denied of iron at the time of autopsy. Effective iron reduction therapy prevents cirrhosis and therefore also reduces the risk of HCC in individuals with hemochromatosis and most certainly contributes to the lower risk of HCC reported in more recent large cohorts of individuals with hemochromatosis *(32, 57, 58)*.

The development of diabetes mellitus in individuals with hemochromatosis and the observation of macrovascular fat and hyperglycogenation in individuals with Wilson's disease suggest that many, if not all, of the mechanisms that contribute to HCC in individuals with NASH may also be contributory mechanisms to the development of hepatocarcinogenesis in both hemochromatosis and Wilson's disease *(52–61)*.

6. AFLATOXIN-ASSOCIATED HCC

Aflatoxin ingestion is high in areas of Southeast Asia and sub-Saharan Africa where grains and rice are a primary food source. The same is the case in China. These same areas typically store grains for prolonged periods and

as a result the grain often becomes contaminated with fungi that produce aflatoxins. These same geographic regions have high rates of HCC wherein a specific p53 mutation (6^{24gt}) is found *(62)*.

Aflatoxin is metabolized to a potential mutagenic intermediate, aflatoxin 8, 9-epoxide, which is normally detoxified by microsomal peroxide hydrolyses and glutathione *S*-transferase *(62)*. Failure to detoxify this mutagenic intermediate has been known to be associated with the identical p53 mutation found in individuals with HCC within these same geographic areas. Moreover, individuals in these geographic regions have an increased rate of inherited isoforms of both microsomal peroxide hydrolyses and glutathione *S*-transferase with either reduced or no activity of these two enzymes *(62)*. Finally, it needs to be pointed out that these same geographic areas have very high rates of HBV infection. Thus, an interaction between the mechanisms leading to hepatocarcinogenesis in individuals with HBV infection described elsewhere in this textbook and those reported for p53 inactivation by aflatoxin and its metabolite may contribute to the increased development of HCC in these regions of the world.

7. ALPHA 1 ANTITRYPSIN DEFICIENCY AND HCC

Alpha 1 antitrypsin deficiency is an autosomal recessive disorder resulting from a single gene defect wherein a defective gene, with either a Z, S, F, or null allele, occurs in either a homozygous or a compound heterozygous state resulting in reduced plasma serine protease activity. As a result, circulating levels of the serine protease, alpha 1 antitrypsin protein, are reduced to 15–60% of normal *(63)* and the protein accumulates in the endoplasmic reticulum of the liver *(64, 65)*. In addition, mitochondria dysfunction and autophagy occur and contribute to the overall hepatic dysfunction and resultant disease progression *(66)*. The underlying pathophysiology is that of an abnormal folding of the protein and its subsequent accumulation in the endoplasmic reticulum that induces an oxidative stress within both the endoplasmic reticulum and mitochondria. The oxidative stress reaction appears to be a consequence of activation of NF-kB, endoplasmic reticular caspase B cell receptor-associated protein 31, and organelle autophagy.

Most clinical cases of alpha 1 antitrypsin deficiency occur in childhood and are manifested as either a transient acute liver failure or a progressive hepatitis resulting in cirrhosis. It is also seen in adults with late onset of portal hypertension and hepatic synthetic dysfunction *(62–72)*.

Hepatocellular carcinoma is common in adults with alpha 1 antitrypsin deficiency after age 50 where it occurs in 31–67% of all cases having cirrhosis with evidence of overt portal hypertension.

More prevalent than homo zygous alpha 1 antitrypsin deficiency is the occurrence of the heterozygous state with either a single Z, S, or F allele and a normal allele. This situation is not directly associated with liver disease

but appears to act as a potentiating factor for liver disease and liver disease progression as well as HCC when it occurs in association with any of a number of other liver disease processes such as HBV, HCV, alcohol, and NASH. The combination of these various other hepatocarcinogenic mechanisms in patients with alpha 1 antitrypsin deficiency may act in an additive or synergistic way and lead to the development a hepatic cancer. As is the case with NAFLD, hemochromatosis, and Wilson's disease, HCC only occurs in those cases that are cirrhotic. Thus, screening and surveillance for HCC need not be instituted until clinical evidence of cirrhosis is present.

8. FAMILIAL INTRAHEPATIC CHOLESTASIS

Each of these diseases is a result of an autosomal recessive disorder resulting in defective hepatocyte canalicular membrane transport.

(A) Progressive familial intrahepatic cholestasis type I was originally described by Byler and has been termed Byler's disease as a result *(73)*. It is a mutation in the FIC-1 gene (ATP8B1) and results in a spectrum of liver diseases ranging from a benign condition with intermittent pruritus with or without jaundice termed benign recurrent intrahepatic cholestasis (BRIC) to severe intractable pruritus, jaundice, and liver failure. Genotype/phenotype correlations have documented more severe mutations in individuals manifesting the PFIC-1 phenotype syndrome than those manifesting the BRIC phenotype, which is characterized by more missense mutations *(74)*. With advanced cholestasis HCC can occur in these cases.

(B) Bile salt export protein (BSEP) deficiency is a result of an autosomal recessive disorder in bile salt secretion due to a defective bile salt export protein which is liver specific unlike that occurring in PFIC-1 *(75)*. Specifically, the disease is due to a mutation in an adenosine triphosphate-binding cassette transporter gene (ABCB11), the principal canalicular transporter of bile acids into bile. Disease severity varies inversely as a function of the degree of BSEP expression. In severe cases, the disorder is termed PFIC-2 and in less severe cases it is termed BRIC-2. Cases of HCC have been reported in the severe forms of BSEP deficiency *(76)*.

(C) Multidrug resistance-3 (MDR-3) deficiency or PFIC-3 is a consequence of a mutant class III multidrug resistance p-glycoprotein identified as MDR-3 (ABCB4) which is responsible for canalicular phospholipid transport *(77)*. Its clinical manifestation is highly variable with clinical onset of disease occurring between ages 1 month to 20 or more years. Unlike the proceeding two conditions that have low levels of gamma-glutamyl transpeptidase despite cholestasis, this disorder is character-

ized by an elevated gamma-glutamyl transpeptidase level. Hepatic cancer can occur in this disorder but its frequency is much less than in the other two forms of familial cholestasis.

9. BILE ACID SYNTHEIC DISORDERS AND HEPATOCELLULAR CARCINOMA

Nine distinct genetic disorders of bile acid synthesis have been identified and characterized clinically *(78)*. All are inherited as an autosomal recessive disorder. They occur as a result of either a specific enzyme deficiency that is unique for normal bile acid synthesis or a disruption in peroxisomal function.

Those due to a defect in bile acid synthesis can be treated medically, but if unrecognized or untreated can progress to cirrhosis and liver failure *(79)*. Liver cancer can occur in these cases but is unusual as liver failure leads to an early death in untreated cases, and autopsies which are likely to identify HCC have rarely been performed in these cases.

The hydrophobic bile acids that accumulate as a result of cholestasis of any cause are known to enhance apoptosis by activating caspases and disrupting the balance between cell cycle renewal and apoptosis. Bile acids also enhance mitogen-activated protein kinase (MAPK) activation dependent on epidermal growth factor receptor activation which enhances cellular regeneration/proliferation mechanisms. The net effect of these two different bile acid-induced mechanisms in individuals with metabolic disease, particularly those metabolic disease with cholestasis, positively affects cell cycle regulation, enhancing cell proliferation and the opportunity for the development of a hepatocellular carcinoma. Both macrophages and neutrophils present in inflammatory tissue can produce ROS and have a cytosolic myeloperoxidase that produces hydrochloride, a powerful oxidant. These cells accumulate within the liver of individuals with various hepatic diseases including essentially every metabolic liver disease and contribute to the overall oxidative stress experienced by the liver.

No therapy exists for those with defective peroxisomal dysfunction. The liver disease in this subset of cases is only a part of the overall disease process wherein the clinical manifestations occur and involve the nervous system and the adrenal glands, as well as the liver.

10. DEFECTS IN CARBOHYDRATE METABOLISM

10.1. Galactosemia

This disorder is characterized by a deficiency of galactose-1-phosphate uridyl transferase. Several different alleles for this disorder have been identified but most cases are due to a single common mutation (Q188R) *(80)*.

The enzymatic defect blocks the metabolism of galactose-1-phosphate and causes hemolysis, jaundice, liver disease, lactic acidosis, renal tubular acidosis, failure to thrive, hepatosplenomegaly, cataracts, and e.coli sepsis particularly in neonates. A single report of HCC in a child with this disorder, who had a transplant, has been reported having not been treated medically, if the child had been treated appropriately with a galactose-free diet clinical liver disease should not have occurred and the hepatic cancer and requirement for a liver transplanted would have been avoided occur *(81)*.

10.2. Hepatic Glycogen Storage Disease

Five different hepatic glycogen storage disorders have been characterized and specifically identified. These are glycogen storage diseases type I, III, IV, VI, and IX. The latter two tend to be mild while the first three, types I, III, and IV, are progressive and can be severe leading to a requirement for liver transplantation *(82)*. Hepatic adenomas and cancer have been reported in types I, III, and IV *(82–86)*. Tumor detection in each disorder is dependent upon imaging procedures.

(i) Glycogen Storage Disease I (GSD-I) is an autosomal recessive disorder with a prevalence of 1/20,000–1/225,000. Glucose 6-phosphate deficiency characterizes GSD-I. The enzyme is expressed on the inner surface of the endoplasmic reticulum. Two distinct enzymatic defects account for this disease. A deficiency of the catalytic compound of the enzyme produces GSD-Ia while a deficiency of the transporter component is responsible for GSD-Ib. The metabolic consequences of the two are identical with the exception that neutropenia occurs with GSD-Ib. Molecular genetic studies are used currently to make the diagnosis and have replaced the older enzymatic activity assays. It is important to note that the latter method of diagnosis can result in a misdiagnosis (failure to identify) of GSD-1B as a result of using frozen tissue that enables the catalytic activity of the endoplasmic reticulum to be assayed and detected but not the transporter component resulting in a false normal result.

Chronic liver disease does not occur in cases of GSD-I but poor metabolic control can result in the development of hepatic adenomas that occasionally degenerate into HCC.

Liver transplantation has been used to treat GSD-I with poor metabolic control with medical measures or as a result of the development of either a hepatic adenoma or a HCC *(51)*.

(ii) Glycogen Storage Disease-III (GSD-III)

Defective glycogen debrancher enzyme characterizes GSD-III. It tends to be milder than type I but also involves muscle and in adults can be manifested with either a severe skeletal myopathy or a cardiomyopathy.

It is an autosomal recessive disorder with a prevalence of 1/20,000–25,000. As was the case with GSD-I, two forms of GSD-III occur. GSD type A involves muscle and liver and represents 85% of the cases. GSD type B accounts for only 15% of cases and involves only the liver.

Cirrhosis can develop in GSD-III unlike type I and liver tumors have been reported in cases with advanced fibrotic liver disease.

(iii) Glycogen Storage Disease IV (GSD-IV)

GSD-IV is an autosomal recessive disorder caused by a deficiency of the glycogen branching enzyme occurring at a rate of 1/20,000–25,000 and results in the accumulation of unbranched glycogen in the liver, heart, muscle, skin, intestines, and nervous systems (both central and peripheral). It typically presents as infantile cirrhosis. HCC has been reported in these cases *(84–86)*.

11. TYROSINEMIA TYPE I

Tyrosinemia type I or hepatorenal tyrosinemia is an autosomal recessive disorder due to a defect in fumarylacetoacetate hydrolase which results in an accumulation of fumarylacetoacetate and maleylacetoacetate *(87)*. It has a prevalence of 1/100,000 worldwide but occurs in specific geographic regions at an increased rate approximately of 1/2000. It presents as acute hepatitis, acute liver failure, or cirrhosis often with a HCC. Apoptosis of hepatocytes is a characteristic feature of the disease *(88)*. The apoptotic signal in tyrosinemia type I appears to be fumarylacetoacetate *(88)*. Both fumarylacetoacetate and malylacetoacetate are alkylating agents that can cause DNA damage. Thus the development of HCC in cases of tyrosinemia type I is due to a combination of DNA and RNA mutagenesis occurring as a consequence of oxidative stress and nucleic acid alkylation *(89–95)*. The oxidative stress is a result of the consumption of antioxidants by malylacetone, fumarylacetone, and succinylacetic acid and succinyl acetone.

The introduction of 2-(2-nitro-4-trichloromethylbenzol)-1,3-cyclohexendrome (NTBC) which blocks tyrosine degradation at 4-hydroxyphenylpyruvate prevents the formation of the alkylating agents fumarylacetoacetate and malylacetoacetate and has greatly altered the natural history of the disease *(96)*. Unfortunately some 10% of cases of tyrosinemia type I do not respond to NTBC therapy and require liver transplantation prior to age 2 if HCC is to be prevented.

12. THE PORPHYRIAS

(A) Acute intermittent porphyria (AIP) is an autosomal dominant disorder resulting from a half normal level of porphobilinogen deaminase. It

is characterized by increased plasma and urinary levels of delta amino levulinic acid and porphobilinogen as well as clinical episodes of recurrent visceral, autonomic, and central neuropathy with abdominal pain. It occurs at a rate of 1/20,000 and is the most common form of porphyria.

(B) Congenital intrahepatic porphyria (CIP) is a very rare autosomal recessive disorder characterized by markedly reduced uroporphyrinogen III synthetase. It has a highly variable age at the time of clinical onset and is characterized by red brown teeth, frequent bacterial infections, and a deposition of iron in the liver and spleen.

(C) Porphyria cutanea tarda (PCT) is an autosomal dominant disorder characterized by reduced levels of uroporphyrinogen decarboxylase. Three different types of the disease are recognized. These are

(1) sporadic (worldwide) occurring at a rate of 1/25,000 in the United States wherein the liver alone is enzyme deficient
(2) familial (autosomal dominant) form that involves enzyme deficiency in the liver and bone marrow
(3) familial (rare autosomal recessive) form that occurs in the liver characterized by sun exposure-induced blistering, dermal scarring, hypo- and hyper pigmentation, hirsutism, and an accumulation of porphyrins in the liver, plasma, and urine. Uroporphyrinogen decarboxylase enzyme activity in the liver can be reduced by iron-dependent oxidative stress induced by alcohol, HCV infection, HIV infection, smoking, and a HFE gene mutation.

(D) Hepatoerythropoietic porphyria (HEP) type II porphyria cutanea tarda is due to a markedly reduced uroporphyrinogen decarboxylase expressed in liver and RBC.

(E) Hereditary coproporphyria (HCP) is autosomal dominant due to reduced activity of coproporphyrinogen oxidase and is characterized by signs and symptoms similar to acute intermittent porphyria but with sun sensitivity manifested by increased urinary coproporphyrins.

(F) Variegate porphyria (VP) in an autosomal dominant disorder characterized by hepatic deficiency of protoporphyrinogen oxidase (PPO). Characterized by neurologic and cutaneous signs and symptoms similar to AIP, it is associated with episodes of severe hyponatremia during attacks.

(H) (G) Erythropoietic protoporphyria (EPP) is an autosomal dominant disorder of ferrochetalase deficiency. It is the third most common form of porphyria. Skin changes are universal with this condition consisting of dermal lichenification and blistering. Protoporphyrins accumulate in the liver and induce a form of biliary cirrhosis.

HCC has been reported to occur in AIP, CIP, PCT, VP, HEP, but not in EPP *(97–105)*.

13. CYSTIC FIBROSIS

Cystic fibrosis is an autosomal recessive disorder that results in the development of abnormal chloride channels and an inability to secrete thin watery secretions in the tracheobronchial tree, intestine, and biliary system. It occurs almost exclusively in Caucasians at a rate of 1/2000–3000 live births. The hepatic manifestations of cystic fibrosis are focal biliary cirrhosis that can become panlobular. The hepatic disease is characterized by cholestasis and inflammation often complicated by episodes of recurrent biliary sepsis. With progressive disease, toxic bile acids accumulate and induce epigenetic alterations that result in defective cell cycle regulation and in rare cases, hepatic cancer in a liver with advanced biliary cirrhosis *(106)*.

The hydrophobic bile acids that accumulate as a result of cholestasis of any cause are known to enhance apoptosis by activating caspases and disrupt the balance between cell cycle situation and apoptosis. Bile acids also enhance mitogen-activated protein kinase (MAPK) activation dependent on epidermal growth factor receptor activation enhancing cellular regeneration/proliferation mechanisms. One net effect of these two bile acid mechanisms in individuals with metabolic diseases particularly those metabolic diseases with cholestasis can affect cell cycle regulation enhancing the opportunity for the development of a hepatocellular carcinoma. Both macrophages and neutrophils can produce ROS and a myeloperoxidase that produces hypochlorite, a powerful oxidant. One or both of these cells accumulate within the liver of individuals with various hepatic diseases including metabolic liver diseases and contribute also to the next oxidant stress experienced by a liver with a metabolic disease.

14. ALAGILLE'S SYNDROME

Alagille's syndrome is an autosomal recessive disorder due to a defect in JAG-1 that results in a paucity of bile ducts and a biliary cirrhosis that can lead to the development of HCC *(107)*. It is characterized by a triangular face, embrotoxin abnormality of the eye, butterfly vertebrae, peripheral pulmonary artery stenosis, and resultant pulmonary hypertension as well as chronic cholestasis.

15. LINKED SIDEROBLASTIC ANEMIA

This disease occurs as a result of a deficient activity of Δ5-aminolevulinic synthetase in the mitochondria of erythroid cells.

As a result ineffective erythropoiesis iron accumulation occurs in the mitochondria of the erythroid cells of the marrow, liver, heart, and joints.

The clinical manifestations of the disease include hepatomegaly, cirrhosis, and HCC, diabetes, hypogonadism, and skin changes similar to hereditary hemochromatosis *(108, 109)*.

16. FANCONI ANEMIA

This is an autosomal recessive disorder characterized by diffuse congenital anomalies, bone marrow failure, and malignancy *(110–113)*. The carrier frequency is 0.5%. Affected individuals are highly sensitive to cross-linking agents and develop numerous chromosomal breaks. The most frequent extra hematologic abnormalities are radial ray defects affecting the distal radius, thumb, hip, vertebrae and knee abnormalities, insulin resistance, and short stature. Liver tumors are common. The roles of androgen therapy, insulin resistance, and DNA repair dysfunction coupled with reduced apoptosis presumably account for the hepatic pathology in this disorder.

17. TYPE II DIABETES MELLITUS

This is a common disorder accounting for >85% of all cases of diabetes mellitus and is typically seen in adults but it also occurs frequently in children especially those manifesting various components of this metabolic syndrome (obesity, hypertension, dyslipidemia, sleep apnea, polycystic ovaries, and gout).

Excessive insulin results in increased growth factor receptor binding protein 2, RAS, RAF, MEK, MAK activation as well as PDK-1 and p70-56 K activation, all of which increase cell proliferation.

These events occurring in conjunction with the adverse effects of hepatic steatosis and the oxidative stress associated with hypertriglyceridemia and free fatty acid increases in the liver and plasma probably account for the mutagenesis which results in the development of hepatocellular carcinoma in cases of type II diabetes mellitus *(114–118)*.

18. HEREDITARY FRUCTOSE INTOLERANCE

Individuals with hereditary fructose tolerance, who survive the neonatal period, can, with repeated fructose challenges, develop fibrosis liver disease and rarely a hepatocellular carcinoma *(119)*.

19. HEREDITARY HEMORRHAGIC TELEANGIECTASIA

This disorder is characterized by vascular lesions in the skin, intestine, and solid organs to include the liver, spleen, kidney, heart, and brain. Typically the disorder presents as recurrent epistaxis. Cardiac failure can occur

with large solid organ artero-venous fistulae. After epistaxis, the major problem is recurrent bleeding necessitating iron and other transfusion therapy. As a result of years of transfusion the development of a blood-borne infection is likely and can result in liver disease and HCC. A rare hepatoma has been reported in patients with this disorder in the absence of a history of hepatitis *(120)*.

20. ADENOSINE DEAMINASE DEFICIENCY

The disorder is a very rare autosomal recessive disorder that results in a severe combined immunodeficiency in children and adolescents. A delayed adult form has been recognized recently and is associated with autoimmune disorders and hepatic dysfunction as well as hepatoma *(121–128)*.

21. STEROID-INDUCED HCC

Estrogens and androgens have both been reported to induce adenomas and hepatomas in the liver. Estrogens are used for the purpose of oral contraception and typically produce adenoma and rarely HCC *(129–131)*.

Androgens are used for their anabolic activity and more often than estrogens produce HCC *(131–135)*.

22. SUMMARY

This chapter discusses the most widely recognized metabolic disorders that are associated with hepatic carcinogenesis. The authors make no assertion that it is all inclusive, rather it presents those that are reasonably well characterized. Other disorders may have random hepatic cancers or liver disease-associated cancers that have yet to be recognized as a frequent occurrence in the disorder as a result of rarity of the metabolic disorder and the low rate of HCC that can occur in them. Thus the recognition of a linkage between the two is very difficult to recognize and quantify.

In all of the disorders recognized and presented herein, the basic metabolic defect includes either a state of oxidative stress or an alteration in cell proliferation or cell death as a downstream consequence of the metabolic defect.

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