Primary Liver Cancers, Part 2: Progression Pathways and Carcinogenesis

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Abstract
Hepatocellular carcinoma (HCC) and primary intrahepatic cholangiocarcinoma (ICC) have been increasing in incidence worldwide and are leading causes of cancer death. Studies of the molecular alterations leading to these carcinomas provide insights into the key mechanisms involved. A literature review was conducted to identify articles with information relevant to current understanding of the etiologies and molecular pathogenesis of HCC and ICC. Chronic inflammatory diseases are the key etiological risk factors for both HCC and ICC, although other diseases play a role, and for many ICs, an underlying risk factor is not identified. Mutations in catenin beta 1 (CTNBB1) and tumor protein 53 (P53) are the main genetic alterations in HCC. Isocitrate dehydrogenases 1 and 2 (IDH1/2), KRAS protooncogene GTPase (KRAS), a RAS Viral Oncogene Homolog in neoroblastoma (NRAS) and P53 are primary genetic alterations in ICC. In both diseases, the mutational landscape is dependent on the underlying etiology. The most significant etiologies and genetic processes involved in the carcinogenesis of HCC and ICC are reviewed.

Keywords
hepatocellular carcinoma, cholangiocarcinoma, liver, carcinogenesis

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Introduction
Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the 2 most common hepatic malignancies. Both have increased in incidence. A number of etiologies predispose patients to the development of either disease. Most commonly, these are processes that induce chronic inflammation and damage to the hepatic parenchyma or the biliary tract. However, HCC and ICC may develop in nondiseased liver. Studies into the carcinogenesis of these malignancies, particularly studies using newer high-throughput technologies, have provided novel understanding of the mechanisms involved.

Hepatocellular Carcinoma
Epidemiology and risk factors for HCC. Liver cancer is much more common in men than in women. In developing countries, it is the second leading cause of cancer death among men. In more developed countries, it is the sixth leading cause of cancer death among men.1 Hepatocellular carcinoma is most prevalent in East and Southeast Asia and Northern and Western Africa, mostly due to the endemic prevalence of hepatitis B viral (HBV) and hepatitis C viral (HCV) infections, which predispose patients to the development of chronic liver diseases and cirrhosis.2-6 In the United States, the age-adjusted incidence rates of liver cancer have more than tripled between 1975 and 2011.1 Increases in HCV infection and possibly also obesity and diabetes are thought to have contributed to this increase.1 In the United States, HCC represents the fastest growing cause of cancer mortality overall and the second fastest growing cause of cancer mortality in females.2

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Hepatocellular carcinoma most frequently arises in a background of chronic liver disease and cirrhosis. The most common risk factors for HCC include chronic infection with HBV and HCV, alcohol abuse, and exposure to aflatoxin B1. Other risk factors include smoking, obesity, and nonalcoholic steatohepatitis (NASH), diabetes, a number of inherited disorders such as hereditary hemochromatosis, and adenomas, particularly β-catenin-activated adenomas as described in part 1. In patients with more than 1 risk factor, the effects are incremental.

Hepatitis B viral infection is known to be directly carcinogenic since patients without cirrhosis often develop HCC. Since it is a DNA virus, HBV is able to integrate into the host genome, replicating and producing HBV X protein, implicated as a key promoter in HCC development. Frequently, HCC has been identified in patients with HBV infection in an absence of liver cirrhosis or significant fibrosis, implicating viral-induced persistent liver injury and regeneration in the initiation of HCC.2-6 By contrast, HCV is an RNA virus that reproduces new viral particles in hepatocellular cytoplasm and does not incorporate into the host genome. In patients burdened with chronic HCV infection, HCC is characteristically diagnosed in liver parenchyma with marked bridging fibrosis and cirrhosis. In patients with long-lasting steatohepatitis and cryptogenic cirrhosis, despite an absence of viral etiology, dysplastic hepatocellular nodules and HCC have been well-documented.2,8-10

Alcohol is one of the oldest known chemical carcinogens associated with the development of HCC and is considered to be the third most common cause of HCC worldwide.11 The risk of liver disease correlates with the amount of alcohol consumed over a lifetime.12 The metabolites of alcohol, acetaldehyde, and reactive oxygen species induce chronic oxidative stress and chronic inflammation and also lead to genomic instability and insufficient repair pathways.7,11,13 In the United States, the risk of liver cancer is increased 2- to 4-fold in patients consuming >60 g/d of ethanol.12 These processes eventually lead to cirrhosis and HCC.7

Aflatoxin B1 is a fungal toxin produced by Aspergillus flavus and A. parasiticus, found on many food products such as nuts, spices, oilseeds, grains, and corn, stored in warm, humid conditions.12 It is the most hepatotoxic and hepatocarcinogenic of the aflatoxins. The risk of HCC is dependent on the duration and dose of exposure. Aflatoxin B1 is directly mutagenic and is associated with a specific P53 mutation14 and mutational activation of oncogenes such as Harvey Rat Sarcoma Viral Oncogene Homolog, HRAS.

Nonalcoholic fatty liver disease is one of the most common causes of chronic liver disease in the United States,11 and it is characterized as associated with obesity, diabetes, and metabolic syndrome with hyperlipidemia. Nonalcoholic fatty liver disease leads to NASH and subsequently cirrhosis. The 5-year risk of developing HCC in NASH is 11.7%.11

Hereditary metabolic disorders also predispose to the development of HCC. These include hereditary hemochromatosis, Wilson disease, α1 antitrypsin deficiency, tyrosinemia, glycogen storage diseases type I and II, and porphyrias.15 The risk of developing HCC in patients with hereditary hemochromatosis is 100- to 200-fold.12

A clear distinction among the various etiologies is that in contrast to HBV and aflatoxin-B1, which affect the genome via viral integration and generation of mutations, respectively, HCV and alcohol induce changes in the liver microenvironment through the development of cirrhosis. Such distinctions and commonalities should assist in the improved design and application of therapies for HCCs in the context of specific etiologies.15

Genetics of HCC. Research studying signaling genes or HCC pathways in cell lines and patient tissue studies have identified multiple key mutations and pathway alterations that occur during hepatocellular carcinogenesis. The most frequent are the Wnt/β-catenin pathway,16,17 and mutations in tumor protein 53 (TP53),14,18,19 Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Protein kinase B (AKT),20 suppressor of cytokine signaling-3 (SOCS3),21 NF-κB,22,23 NF-κB essential-modulator (NEMO)/Inhibitor of nuclear factor kappa-B kinase subunit gamma (IKKγ),24 P16,25,26 Myelocytomatisis Viral Oncogene Homolog (MYC),27,28 and human hemochromatosis (HFE).29,30 Abnormally activated Wnt-β-catenin and Hedgehog pathways are causes of altered cellular proliferation in HCC, with aberrant accumulation of β-catenin in HCC cell nuclei.17 Furthermore, aberrant Wnt signaling has been implicated in the malignant transformation of preneoplastic hepatic adenomas. Investigation of TP53 by immunohistochemistry, DNA sequencing, and allelic deletion analysis has identified loss of P53 heterozygosity in moderately and well-differentiated HCC tumors, irrespective of serological markers of HBV and HCV infections.19 In particular, P53 protein accumulation, with a GTTval → GCTala mutation at codon 274, and a silent mutation (ACCthr → ACThr) at codon 140 of the P53 gene were highlighted by these findings.18

In PI3K/AKT-modulated HCC pathway, activation of the PI3K/ AKT kinase functions blocks the growth inhibitory activity of CCAAT-enhancer-binding proteins (C/EBP)-α through PP2A-mediated dephosphorylation of C/EBP-α on Ser 193, blocking C/EBP-α from interacting with and inhibiting Transcription factor in higher eukaryotes (E2F).20 These findings provide a molecular basis for the development of HCC, whereby activated PI3K/AKT pathway antagonizes C/EBP-α-mediated inhibition of hepatocellular proliferation. Deletion or silencing of the SOCS3 gene in the hepatocytes protects against hepatocellular apoptosis and promotes the activation of Signal transducer and activator of transcription 3 (STAT3), contributing to enhanced hepatitis-induced carcinogenesis.21 Moreover, signal transducer NF-κB enhances chemical exposure–related hepatocarcinogenesis via sustained c-Jun N-terminal kinase-1 (JNK1) activation and acts as a tumor promoter in inflammation-associated carcinogenesis.22 In hereditary hemochromatosis patients, penetrance of the HFE C282Y homozygous genotype has been determined to contribute to HCC development in male patients.29

Next-generation sequencing (NGS) studies have provided greater insight and depth into the understanding of the genetics of HCC. P53 and CTNBB1 are consistently found to be the most
frequently mutated tumor suppressor gene and oncogene, respectively, involved in HCC carcinogenesis, and mutations in one are mostly exclusive of these mutations of the other. Distribution of these mutations is related to the underlying pathology. For example, mutations in CNB1 occur more frequently in HCV-associated HCC and nonviral-related HCC compared to HBV-related HCC. In contrast, P53 mutations occur more frequently in HBV-associated HCC compared to HCV and in viral-associated HCC. Other driver mutations include Kelch-like ECH-associated protein 1 (KEAP1), Homo sapiens chromosome 16 open reading frame 62 (C16orf62), (MLL4), and A signaling GTPase of the Rho family GTPase (RAC2).22

As a result of NGS studies, inactivating mutations in genes encoding proteins in chromatin remodeling have been identified as being frequent in HCC. ARID2 is frequently reported. Other chromatin remodeling genes include ARID1A, ARID1B, MLL, and MLL3, other genes include NMLX1, NLRP1, RPS6KA3, NFE2L2, and IRAF.31 Genetic alterations in chromatin remodelers were observed in 50% of HCC, making these very common mutations.31

Separate studies of HBV integration into the human genome using PCR-based technology identified certain loci that are more frequently involved. The first HBV integration event was identified at the human TERT gene locus. Other genes including FAR2, ITPR1, IRAK2, MAPK1, MLL2, and MLL4 have been discovered with recurrent HBV integration events in more than 1 tumor sample31 identified as integration sites.31 Next-generation sequencing studies have identified MLL 4 and ANGPT1 as integration sites of HBV. Other identified integration sites include the gene loci for CCNE1, ROCK1, and SENP5. Integration into CCNE1 drives aberrant cell cycle control in HCC.33

Pathways altered in HCC. These NGS studies have defined the pathways most frequently involved in HCC carcinogenesis.34 Alterations in telomere maintenance are the most frequent, either due to mutations in the TERT gene or insertion of the HBV viral DNA into the TERT gene loci. The Wnt/β-catenin pathway is the main or most frequently altered pathway in HCC. In addition to β-catenin (CTNNB1), Axis inhibition protein 1 (AXIN1) is another gene frequently mutated that is involved in this pathway. Mutations in genes controlling cell cycle regulation including P53, CKDN2A, Axis inhibition protein (ATM), and Interferon Regulatory Factor 2 (IRF2) have been identified. Genes involved in chromatin remodeling, MLL and ARID gene families, as described, are the next most frequent. Another pivotal signaling pathway identified by NGS was the activation of the nuclear factor erythroid 2-related factor. We suggest keep CUL3 and NRF2 here (NRF2/KEAP1) pathway, which is involved in oxidative stress. NRF2 (coded by NFE2L2) is a transcription factor that is physiologically degraded by the proteasome in a complex with KEAP1 and Core component of multiple cullinRING-based BCR E3 ubiquitin-protein ligase complexes (CUL3). Finally, activating mutations of PI3K, Fibroblast growth factor 19 (FGF19) amplification, and inactivating mutations of RPS6KA3 are also recurrent genetic alterations in HCC, leading to a constitutive activation of PI3K/Akt/mTOR and Ras/Raf/MAP kinase pathways.34 Figure 1 illustrates the pathways involved in HCC carcinogenesis and the corresponding mutations and associated frequencies.

Hepatic stem cell theory. Hepatic stem cells are speculated to be the origin of HCC based on ongoing investigations. The current theory is that HCCs originate from deregulated hepatic stem cell proliferation initiated following chronic stimulation by viral infection or other insults; certain cells in HCC and dysplastic liver nodules have been confirmed to carry molecular prints suggestive of such stem cells.35,36 The superimposed injury by alcohol usage, chemical, or medication/drug effects and subsequent advanced fibrosis, combined with other possible contributing factors such as hemochromatosis and Wilson disease, have significantly increased the risk of HCC development. The increasing prevalence of metabolic syndromes has resulted in an enlarging population of patients with NASH, leading to chronic liver injury, bridging fibrosis, and cirrhosis and contributing to HCC development.

Cholangiocarcinoma

Epidemiology and Risk Factors

It is now accepted that intrahepatic cholangiocarcinoma (ICC) accounts for 10% to 20% of primary intrahepatic malignancies.37-39 The incidence of ICC has risen steadily worldwide in recent years40,41; during a 30-year period, the incidence of ICC increased to 165% in the United States.37,42 This increased incidence is independent of tumor size or tumor stage and is unlikely to be secondary to earlier detection,42 but rather, in fact, reflects a true increase in the incidence of ICC.37,42 Although the exact cause of this increase is uncertain, it could be closely related to the increasing incidence of ICC risk factors.

The majority of patients with ICC have no known predisposing risk factors at the time of presentation.41,43 Processes that produce chronic inflammation, bile stasis, and cirrhosis are predisposing factors for the development of cholangiocarcinoma. These include primary sclerosing cholangitis (PSC), intrahepatic lithiasis, fluke infestation, profession-related chronic exposure to chemicals, and pancreatobiliary congenital maljunction.44-46

Primary sclerosing cholangitis is the most common risk factor for ICC in Western countries, but only 10% of ICCs are attributed to PSC. Patients with PSC have a 5% to 20% lifetime risk to develop cholangiocarcinoma.45 Patients with PSC present at an earlier age, usually in the third or fourth decade,48,49 whereas it typically presents in the seventh decade in patients without PSC.49 Alcohol consumption increases the risk of developing PSC.50

Patients with bile duct cystic disorders also present at an earlier age.49 Patients with Caroli disease and types I and IV biliary cysts have a 30-fold risk of cholangiocarcinoma. Hepatolithiasis is associated with a 6- to 50-fold increased risk of cholangiocarcinoma.37 Infection with the liver flukes, Opisthorchis viverrini and Clonorchis sinensis, is prevalent in Southeast Asia and is a primary risk factor for cholangiocarcinoma in that part of the world.
The relationship between HBV and HCV infection and cirrhosis with the development of ICC has more recently been established.\textsuperscript{47,51,52} Clarification of the associations between ICC and the risk factors is crucial. In most cases, the tumors are at advanced stages at the time of diagnoses, which severely restricts treatment options. Unfortunately, the

\textbf{Figure 1.} Pathways involved in hepatocellular carcinogenesis. Signalling Pathways recurrently mutated in HCC are shown in the right panel. Oncogenes are indicated in red and tumor suppressor genes in blue with percentage of alterations. Reproduced with permission from Nault, JC, and Zucman-Rossi, J. Genetics of Hepatocellular Carcinoma: The Next Generation. Journal of Hepatology. Publisher: Elsevier Date January 2014.

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majority of patients develop ICC in the absence of identifiable risk factors.

Exposure to thorotrast has been strongly associated with the development of ICC. Exposure to other potential carcinogens within certain industries, such as the auto, rubber, and chemical industries, have also been associated with ICC, although the association is less strong. A meta-analysis identified obesity, diabetes mellitus type II, and alcohol use as risk factors for ICC, probably because all of these induce inflammation in the liver and may lead to cirrhosis. In addition, studies have associated variants of genes that regulate DNA repair, inflammation, and carcinogen metabolism with ICC development.

**Molecular Alterations**

A spectrum of genetic alterations involving oncogenes, tumor suppressor genes, and chromatin-modifying genes, are involved in cholangiocarcinogenesis, and newer technologies are aiding the discovery of the mechanisms involved in cholangiocarcinogenesis. The most frequent genetic alterations identified to date include KRAS, BRAF, NRAS, TP53, SMAD/ DPC4, p16 (INK4a) and ARID1A, BAP1, and PBRM1, and IDH1/2. Genetic and epigenetic alterations cause the activation of these oncogenes and/or loss of these tumor suppressor gene functions. Next-generation sequencing has shown that the mutational landscape differs according to the etiology and anatomic location of the cholangiocarcinoma.

A caveat in some of the older studies is that perihilar cholangiocarcinoma (PCC) was misclassified as ICC and this needs to be considered when evaluating data from retrospective molecular profiling studies.

The most frequently identified mutation has been KRAS with hotspot mutations at codon 12. Mutations in P53 were reported in up to 21% of cholangiocarcinomas (CCA) in a review of 10 studies, comprising 229 patients with CCA from Europe, Asia, and the United States. Mutations in IDH1 have been detected more frequently in ICC than in extrahepatic cholangiocarcinoma (ECC), occurring in 28% of ICC compared to cholangiocarcinomas at other locations (7%). A subsequent analysis of 62 cholangiocarcinomas detected isocitrate dehydrogenases 1 (IDH1) mutations in only ICC. Mutations in the other genes are less frequent.

Zhu et al analyzed the incidence and prognostic significance of mutations associated with ICC using nucleic acids extracted from 200 resected ICC tumor specimens using a mutational profiling panel that queried 150 hotspot mutations of 15 known cancer genes. In their study, a majority of the tumors did not have a mutation identified, supporting a need for broad-based gene mutational profiling in patients with ICC. IDH1 and KRAS were the most frequently identified. Other genetic mutations identified in very low frequency included v-Raf murine sarcoma viral oncogene homolog B (BRAF), IDH2, PI3K, NRAS, TP53, MAP2K1, CTNNB1, and Phosphatase and tensin homolog (PTEN). Concurrent mutations in KRAS and BRAF and IDH1 and KRAS were not identified. Putra et al compared ICC to ECC and found that ICC harbored IDH1 and NRAS mutations, whereas KRAS, P53, and BRAF mutations were found in ECC, confirming a difference in the mutational landscape according to location of the cholangiocarcinoma.

A recent study using integrative molecular analysis was able to distinguish ICC into those associated with inflammation and having alterations in the inflammatory pathways and those not associated with inflammation. The inflammation class included activation of inflammatory pathways, overexpression of cytokines, and activation of STAT3. The proliferation class included activation of oncogene signaling pathways involving RAS, MAPK, and MET.

There are differences in the genetic alterations between ICCs arising in cirrhotic livers and those arising in noncirrhotic or normal liver. Jang et al have utilized high-throughput mass spectrometry-based platform to decipher molecular alterations in ICCs and to compare the mutational profiles between 43 ICCs with normal liver and 38 with chronic advanced liver diseases. They detected 47 mutations in 11 genes in 38 (46.9%) of 81 cases; the investigation indicated that the most commonly mutated gene was KRAS (11/81, 13.6%), followed by MLH1 (7/81, 8.6%), NRAS (7/81, 8.6%), GNAS (6/81, 7.4%), and epidermal growth factor receptor (EGFR; 6/81, 7.4%). In addition, BRAF, Adenomatous polyposis coli (APC), PI3K, CDKN2A, PTEN, and P53 mutations were documented in <5% of cases. Their research suggests that the overall mutation rate of ICCs with chronic advanced liver disease (15/38, 39.5%, 95% confidence interval [CI]: 23.9-55.0) was lower than that of ICCs with normal liver (23/43, 53.5%, 95% CI: 38.5-68.3). Interestingly, ICCs with chronic advanced liver disease showed significantly higher EGFR mutation rates (5/38, 13.2% vs 1/43, 2.3%) and lower mutation rates of KRAS (3/38, 7.9% vs 8/43, 18.6%), MLH1 (2/38, 5.3% vs 5/43, 11.6%), and GNAS (1/38, 2.6% vs 5/43, 11.6%), compared with those in ICCs with normal liver. Mutations in PI3K, PTEN, CDKN2A, and P53 were harbored only in ICCs with normal liver, whereas KRAS (P = .0075) or GNAS mutations (P = .0256) were associated with poor overall survival in all patients with ICC. These distinct mutational profiles of ICCs additionally suggest different carcinogenic pathways and illustrate that different therapeutic strategies should be developed for targeted therapy in ICCs.

One important signaling involved in carcinogenesis of cholangiocarcinoma is PI3K/AKT/PTEN pathway, where PI3K functions under the modulation by its upstream regulators especially RAS oncogene. RAS operates in a signaling complex with multiple activators and effectors. PI3K is one of the most important effectors of RAS, regulating multiple functions including cell growth, cell cycle entry, cell survival, cytoskeleton reorganization, and apoptosis. Loss of phosphatase function of PTEN due to either protein loss or its suppression by the upstream molecules will lead to constitutive activation of the PI3K/Protein kinase B (AKT) signaling in cholangiocarcinoma, including downstream the mechanistic target of rapamycin (mTOR), heralding a worsened prognosis for the patients; inhibition of PI3K/AKT signaling effectively suppressed proliferation and invasive behavior of cholangiocarcinoma tumor
cells, indicative of the potential therapeutic value of PI3K and/or AKT inhibitor(s).

In parallel, deregulation of protein kinase signaling by actively mutated RAS and RAF oncoproteins has been confirmed in two-thirds of ICC, illustrating the tight connection between these molecules and carcinogenesis of ICC. Furthermore, Wnt pathway molecules including cyclin D1, c-Myc, and urinary-type plasminogen activator receptor contribute to cholangiocarcinogenesis and are overexpressed in most tumors. Specifically, immunohistochemical studies of β-catenin demonstrated positive staining in cytoplasm and/or nucleus in 58.3% ICC, and the overexpression of cyclin D1 was statistically correlated with that of β-catenin, illustrating abnormally activated Wnt signaling pathway in ICC. Activation of Wnt signaling pathway, together with inactivation of P53, led to more aggressive ICC behavior in animal models comparable to human ICC progression. Importantly, aberrant activation of this signaling pathway has been associated with both carcinogenesis in HCC and ICC.

Variations in the tumor suppressor P53 and murine double minute 2 (MDM2) antioncogenes are closely connected to genetic susceptibility to biliary neoplasms. P53 and its negative regulator MDM2 cooperate in modulating basic cell functions such as cell cycle control and apoptosis; the errors in their expressions and functions contribute to biliary carcinogenesis, displayed by cholangiocarcinoma formation in animal models carrying germline P53 mutants. A convincing association between functional variation in P53 and MDM2 and susceptibility to ICC in human has not been completely established.

Hypermethylation of genes tightly modulates cellular fate by controlling proliferation, migration, cell cycle, DNA repair, angiogenesis, and apoptosis and is involved in carcinogenesis. It has been accepted that there is a complex interplay of genetic and epigenetic alterations that accumulate in precancerous tissues and culminate in the development of full-blown carcinoma. Not surprisingly, methylation of P16 and its inactivation has been detected as an early event in near 90% of cholangiocarcinoma, and loss of P16 expression has been correlated with poor patient outcomes.

**Inflammation and Cholangiocarcinoma Carcinogenesis**

Carcinogenesis in the biliary tract following chronic inflammation is a clinically acknowledged event. Chemical injury, inflammation, stones, or infections are all proven risk factors. For example, pancreaticobiliary maljunction causes the reflux of bile and pancreatic secretions, injuring the mucosa in the gallbladder and common bile duct. A variety of inflammatory modulators and molecules produced or activated during chronic inflammation act to activate oncogenic signaling in biliary mucosa. A separate class of inflammatory ICC has been demonstrated, with activation of inflammatory pathways. Chronic inflammatory processes induce productions of multiple cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), transforming growth factor-β (TGF-β), and platelet-derived growth factor; these molecules are involved in carcinogenesis of cholangiocarcinoma by affecting biliary epithelial function and proliferation. Multiple important signaling pathways have been identified to be involved in biliary carcinogenesis, which include TGF-β/Smad, IL-6/STAT-3, PI3K/AKT, Wnt, RAF/MEK/MAPK, and Notch cascade. For example, IL-6 induces antipoptotic protein myeloid cell leukemia 1 (Mcl-1) via phosphorylation of signal transducers and activators of transcription 3 in cholangiocarcinoma. Gankyrin activates IL-6/STAT3 signaling by suppressing Rb. Silencing of suppressor of cytokine signaling 3, which controls the IL-6/STAT3 signaling, further contributes to sustained IL-6/STAT3 signaling and enhanced Mcl-1 expression in cholangiocarcinoma. Epigenetic regulation by IL-6 contributes to cholangiocarcinoma progression via affecting promoter methylation and gene expression in growth regulatory pathways, including that of EGFR.

A well-studied example illustrating the intimacy between biliary carcinogenesis and chronic inflammatory processes is PSC and ICC. Primary sclerosing cholangitis, which is an idiopathic and progressive cholestatic liver disease characterized by inflammation, concentric fibrosis, and obliteration of the intrahepatic and extrahepatic bile ducts, with concomitant ductal injury and ductal disappearance, is tightly connected to cholangiocarcinoma. Multiple biliary molecules have been uncovered to play distinct roles in cholangiocarcinoma carcinogenesis. One of them is S100A9, a calcium-binding protein and a marker for disease activity in PSC.

Interestingly, innate natural killer immunity is associated with protection against cholangiocarcinoma carcinogenesis in patients with PSC, although the involved molecular mechanism has not been clearly established; presumably its interaction with the major histocompatibility complex (MHC) class I polypeptide plays a key role in this process.

**Cholangiocarcinoma Progression Pathways**

Cholangiocarcinoma follows a stepwise carcinogenesis process through a precursor lesion: biliary intraepithelial neoplasia (BilIN). Mutations of KRAS have been confirmed in more than 30% of BilIN lesions, occurring as an early signaling event during the progression of BilIN to cholangiocarcinoma, including those BilIN arising from large bile ducts, whereas P53 overexpression has been proved to happen afterward. Of notice, KRAS mutation and that of BRAF are identified to be mutually exclusive; however, both are associated with a higher cholangiocarcinoma stage at the time of surgical resection and show an increased risk of lymph node metastasis, as well as associated shortened patient survival.

**Summary**

Worldwide, the incidence of primary HCC and ICC has been steadily increasing. Current investigations have shed light on the etiologies and molecular pathogenesis of HCC and ICC.

Chronic inflammatory diseases remain major risk factors for both HCC and ICC, and additional culprits also contribute to disease initiation and progression, although frequently in ICC cases a definitive underlying risk factor cannot be determined with certainty. Among those identified or discovered risk factors, PSC and ICC stand out for their strong association with chronic inflammation.
factors, mutations in \textit{CTNBB1} and \textit{P53} have been proven to be the main genetic alterations in HCC; in parallel, primary genetic alterations involving \textit{IDH1/2}, \textit{KRAS}, \textit{NAS}, and \textit{P53} genes have been implicated in cholangiocarcinogenesis. The mutational profiles are closely related to the underlying etiologies in both HCC and ICC, and they also impact the disease progression; some of these affected molecules could potentially serve as therapeutic targets in the future in our combat against these lethal neoplasms.

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