Trends in Molecular Medicine

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Review

Genomic Profiling and Metabolic Homeostasis in Primary Liver Cancers

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Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA), the two most common primary liver cancers, represent the second most common cancer-related cause of death worldwide, with most cases being diagnosed at an advanced stage. Recent genome-wide studies have helped to elucidate the molecular pathogenesis and genetic heterogeneity of liver cancers. This review of the genetic landscape of HCC and iCCA discusses the most recent findings from genomic profiling and the current understanding of the pathways involved in the initiation and progression of liver cancer. We highlight recent insights gained from metabolic profiling of HCC and iCCA. This knowledge will be key to developing clinically useful diagnostic/prognostic profiles, building targeted molecular and immunologic therapies, and ultimately curing these complex and heterogeneous diseases.

Heterogeneity of Primary Liver Cancers

Primary liver cancers represent a heterogeneous group of malignant tumors with distinct histological and molecular features. HCC accounts for 90% of all cases of primary liver cancer and may arise in conjunction with one or more risk factors, including hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, adeno-associated virus type 2 (AAV2) infection, alcoholic cirrhosis, non-alcoholic steatohepatitis (NASH), obesity/diabetes, autoimmune hepatitis, consumption of **aflatoxin B1** (AFB1; see Glossary) or contaminated foods, and exposure to other chemical carcinogens [1–3]. The second most common liver cancer is iCCA, which shares many common risk factors with HCC [3,4] (Table 1). iCCA and HCC share some risk factors; however, several unique risk factors contribute to the distinct molecular pathobiology of iCCA and are discussed in Box 1. Other primary liver cancers, including **fibrolamellar hepatocellular carcinoma** (FLC) and the pediatric neoplasm **hepatoblastoma**, account for <1% of cases [5,6].

Chronic inflammation stemming from chronic liver disease, including chronic viral hepatitis, advanced hepatic fibrosis and cirrhosis, is thought to be central to HCC pathogenesis. Cirrhotic livers precede HCC in 80–90% of patients and are considered to be precancerous lesions [7]. Malignant transformation to HCC can occur without cirrhosis in some cases, for example in **hepatocellular adenoma** (HCA), a rare benign liver tumor [8], and HCC with AAV2 infection [9]. Furthermore, chronic liver damage seems to be sufficient to drive hepatocarcinogenesis in the presence of NAFLD, NASH, or HBV infection [7,10,11]. Several cholangiocarcinoma-specific risk factors have been established, including parasite infections, primary sclerosing cholangitis, biliary duct cysts, and hepatolithiasis [4]. However, most cases of iCCA outside Southeast Asia, where biliary infestation with flukes is prevalent, are not associated with these cholangiocarcinoma-specific risk factors [4]. Additional risk factors for iCCA include cirrhosis, HBV/HCV hepatitis, alcohol abuse, diabetes, and obesity, all of which have also been reported to be risk factors for HCC, suggesting that there may be a common pathogenesis to all primary

Highlights

Comprehensive genomic profiles of HCC and iCCA have revealed the molecular events driving and supporting liver cancers and defined cancer subtypes with distinct prognoses.

TERT promoter mutations are the most frequent alterations in HCC as well as in cirrhosis. *TP53* is frequently mutated in HCC and iCCA, especially in hepatitis B virus-associated liver cancers. Genetic alterations in the WNT/β-catenin signaling pathway cooperate with other signaling pathways to facilitate liver cancer initiation and progression.

TGF-β signaling has a context-dependent role in HCC initiation and progression. Defective TGF-β signaling impairs DNA damage repair, while overactive signaling facilitates immunosuppression.

Obesity contributes to the burden of HCC and iCCA. Obesity-driven dysfunctional adipose tissue and gut dysbiosis promote liver cancer initiation and progression via immune cell infiltration, inflammatory signaling, and metabolic alterations.

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Table 1. Major Clinical and Molecular Features of HCC and iCCA[a]

Cancer types/ characteristics	HCC	iCCA
Etiology and risk factors	Cirrhosis HBV, HCV, or AAV2 infection Alcohol abuse NAFLD, NASH, obesity, diabetes α1-Antitrypsin deficiency, hemochromatosis Aflatoxin B1, aristolochic acid HCA transformation (estrogen-based oral contraceptive use)	Biliary infestation with flukes Primary sclerosing cholangitis Biliary duct cysts Hepatolithiasis Cirrhosis HBV/HCV infection Alcohol abuse Diabetes and obesity
Affected Demographic	Adults, >45 years (95%); 50–59 years (54%) [22]	Adults 23–85 years median: 61 years [12,14]
Diagnostic Tests	Abdominal ultrasound Radiologic imaging (CT, MRI) Liver biopsy Serum biomarker: AFP	Radiologic imaging (CT, MRI, ^{18}FDG PET-CT) Endoscopic ultrasonography Liver biopsy Serum biomarker: CA19-9
Therapeutic options	Surgical resection Liver transplantation Locoregional therapy (TACE, RFA, TARE, MWA) Targeted therapy: sorafenib and regorafenib Immunotherapy: nivolumab (anti-PD-1 monoclonal antibody)	Surgical resection Chemotherapy (gemcitabine and cisplatin) Locoregional therapy (TACE, RFA, TARE, MWA) No targeted therapy
Prognosis	Overall 5 year survival: <12% [3] After curative surgery: 50–80%, median >60 months After palliative treatment: median 11–26 months [1]	Overall 5 year survival: (15–40%) [12] After curative surgery (T1–2 disease): 20–24%, median 26 months After palliative treatment (T3–4 disease): median 12–15 months [12,14]
Cellular origin	Mature hepatocytes Hepatic stem/progenitor cells	Mature hepatocytes Mature cholangiocytes Hepatic stem/progenitor cells
Major molecular features	*TERT* promoter mutations (44%) *TP53* mutations (31%) *CTNNB1* mutations (27%)	*FGFR2* gene fusions (25%) *IDH1/2* mutations (9–10%) *TP53* (39–44%) and *SMAD4* (16–19%) mutations in fluke-infected iCCA
Refs	[1,3,22]	[12,14]

[a]Abbreviations: AFP, α-fetoprotein; CA19-9, carbohydrate antigen 19-9; CT, computed tomography; FDG, fluorodeoxyglucose; MRI, magnetic resonance imaging; MWA, microwave ablation; PD-1, programmed cell death protein-1; PET, positron emission tomography; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TARE, transarterial radioembolization.

liver tumors [12–14]. Recently, cholangiocytes have been shown to act as facultative liver stem cells during impaired hepatocyte regeneration and also generate hepatocytes, suggesting that HCC is possibly derived from cholangiocytes during liver injury and parenchymal cell regeneration [15]. Therefore, hepatocytes, biliary epithelial cells (cholangiocytes), and adult stem and progenitor cells (oval cells) have been suggested as potential cells of origin for both HCC and iCCA (the details of the cellular origin of HCC are reviewed in [5,6]). Furthermore, a recent study that integrated genomics, **transcriptomics**, and **metabolomics** from hundreds of HCC and iCCA tumor samples identified common molecular subtypes across both primary liver cancer types, particularly among Asian patients, supporting the notion that primary liver cancers may be considered as a continuum of overlapping neoplasms rather than entirely distinct entities [16,17]. Most recently, a consensus terminology was developed for combined HCC and iCCA (cHCC-CCA), a specific subtype of primary liver cancers that shares common characteristics

Glossary

Aflatoxin B1 (AFB1): a fungal carcinogen present in mycotoxin-contaminated food supplies. AFB1-related HCC is highly associated with a specific hotspot mutation at R249S in the tumor suppressor gene *TP53*.
Dysbiosis: a microbial imbalance or maladaptation on or inside the body, such as an impaired microbiota.
Fanconi anemia genes: a group of genes that mediate DNA damage response. Mutations in these genes lead to Fanconi anemia, a rare genetic disease.
Fibrolamellar hepatocellular carcinoma (FLC): a rare liver cancer that accounts for <1% of primary liver cancers, affecting primarily young adults (10–35 years of age) with no underlying chronic liver disease. The most remarkable genomic characteristic of FLC is a somatic, *DNAJB1–PRKACA* fusion transcript on chromosome 19.
Gain-of-function: a mutation that confers new or enhanced activity on a protein.
Hepatoblastoma: the most common pediatric liver tumor, comprising 1% of total pediatric malignancies with an annual incidence of 1.5 cases per million. Hepatoblastomas are embryonal neoplasms that are most commonly diagnosed during the first 3 years of life. The Wnt signaling pathway gene *CTNNB1* is the most frequently mutated gene in hepatoblastoma.
Hepatocellular adenoma (HCA): a rare benign liver tumor, long associated with use of estrogenbased oral contraceptives. HCA develops in the absence of cirrhotic liver disease. Development of HCA into HCC likely entails a multistep processes, although the classic histologic precursor features seen in premalignant cirrhotic lesions have not yet been observed in HCA-derived HCC.
Immune class: a malignancy classification system based on expression of immune-related gene products. A recent study established immune classes within HCC, including exhausted and active immune subclasses [66]. Approximately 25% of HCCs demonstrated a high degree of immune infiltration with high expression levels of PD-1/PD-L1,

Box 1. Distinct Genetic Alterations in iCCA

Although iCCA and HCC share some risk factors such as HCV and HBV infection and liver cirrhosis, several unique risk factors contribute to the distinct molecular pathobiology of iCCA [105–108]. iCCA samples from patients infected with the *Opisthorchis viverrini* fluke, a parasite with high prevalence in Southeast Asia, were frequently found to contain *TP53* (39–44%) and TGF-β component *SMAD4* (16–19%) mutations. Conversely, *BAP1* (BRCA-associated protein 1) (10–32%) and *IDH1/2* (9–10%) were more frequently mutated in non-fluke-related iCCA, suggesting a specific fluke-associated pathogenesis in iCCA [5,109]. Strikingly, no mutations in β-catenin (0% vs 27% in HCC) and only rare mutations in the *TERT* promoter (0–2% vs 40% in HCC) were found in iCCA from patients with hepatitis. Instead, frequent *KRAS* mutations (16–20% vs 1% in HCC) were found in iCCA, demonstrating the significantly different molecular features of iCCA and HCC [27,73,106,107,110–112]. In addition, *IDH1/2* mutations were more frequent in iCCA (9–10%), while they were rare in HCC (1%) [73,107,112]. Gain-of-function *IDH1/2* mutations lead to the accumulation of 2-hydroxyglutarate, which has been shown to alter the cellular metabolic state and lead to histone and DNA hypermethylation, and in turn drive oncogene expression [73,113].

Another prevalent genetic alteration found in iCCA is a fibroblast growth factor receptor 2 (*FGFR2*) gene fusion. Several *FGFR2* gene fusion products have been reported, including *FGFR2–BICC1* [114], *FGFR2–KIAA1598* [115], *FGFR2–TACC3* [115], *FGFR2–AHCYL1* [106], *FGFR2–MGEA5* [108], and *FGFR2–PPHLN1* [107]. These *FGFR2* gene fusions are uniquely and commonly found in iCCA (25% in iCCA vs 0% in HCC) [105–108], suggesting that *FGFR2* fusion products are potential biomarkers for iCCA diagnosis. Treatment with FGFR2 kinase inhibitors effectively suppressed iCCA cell transformation, and antitumor activity of an FGFR2 inhibitor was noted in a patient with an *FGFR2* fusion [106,108]. Currently, two early-phase clinical trials targeting FGFR2 in advanced iCCA are under way [5,109]. Importantly, *FGFR2* gene fusions represent actionable alterations which can be inhibited by targeting FGFR2 kinase activity. These gene fusion products, which are not found under physiologic conditions, also have a unique chemical structure compared with wild-type *FGFR2*, opening the possibility of fusion-specific therapies in the same way as the drug imatinib selectively targets the *BCR–ABL* fusion gene in chronic myelogenous leukemia [116]. It may be possible to design immunotherapies to train the immune system to recognize this abnormal protein in iCCA.

with poorly differentiated HCC and iCCA with stem cell traits [18], in recognition of the complicated heterogeneity of primary liver cancers.

Advances in antiviral therapy for HCV infections now permit virus clearance in most patients; however, treatment of HBV remains challenging given the lifelong persistence of the virus in most patients [19]. While the incidence of HCC worldwide is generally stable, the incidence of iCCA has been steadily increasing worldwide, and affected patients still have a poor prognosis and limited therapeutic options, largely owing to the advanced stage at diagnosis when surgical control and locoregional approaches in the early stages are no longer possible [20–22]. Unlike the highly targeted molecular therapies available for patients with breast cancer, lung cancer, or melanoma, the only US FDA-approved targeted molecular therapies for patients with advanced HCC are sorafenib and regorafenib, drugs which inhibit multiple kinases [3]. These non-specific inhibitors offer a response rate of <5% and a median response duration of <3 months (patients treated with sorafenib or regorafenib vs patients received placebo) [23,24]. The FDA recently approved an anti-PD-1 monoclonal antibody, nivolumab, for HCC patients previously treated with sorafenib based on results from a Phase I/II clinical trial (NCT01658878), providing an additional treatment option for patients with advanced-stage liver cancer [25]. In contrast to HCC, there are no established first-line locoregional therapeutic options for patients with non-resectable iCCA [26]. Therefore, the identification of key driver genes and signaling pathways in HCC and iCCA progression and the development of novel approaches for modeling HCC and iCCA pathogenesis will be essential to enable improved early detection and effective focused treatment of these cancers. Integration of these findings and understanding of the key pathways driving HCC and iCCA might enable improved research and clinical studies to address this pressing but unmet need for improved therapies. In this review we focus on HCC and iCCA, and highlight the most recent findings from genomic profiling and the current understanding of the pathways involved in the initiation and progression of HCC and iCCA.

suggesting that these HCC cases may be well-suited for PD-1 modulating immunotherapy [66].

Late TGF-β signatures: the early and late TGF-β signatures were initially established using transgenic TGF-β receptor 2-knockout mouse models and subsequently validated in human HCC [68]. Tumors from patients bearing a late TGF-β signature showed significantly shortened mean survival time compared to patients with an early TGF-β signature [68].

Metabolomics: a systematic study of chemical processes involving metabolite profiles.

Nonsynonymous somatic mutations: a change in the genetic structure that is not inherited from a parent, and also is not passed to offspring. There are several common types of nonsynonymous somatic mutations, including missense mutations (point mutations that result in the substitution of a different amino acid in the encoded protein) and nonsense mutations (substitution of an original amino acid codon for a stop codon, causing termination of the protein product).

The Cancer Genome Atlas (TCGA): a multi-institutional effort to understand the molecular basis of cancer through genome analysis technologies, including large-scale genome sequencing technologies.

Transcriptomics: a systematic study of transcriptomes, including mRNA, rRNA, tRNA, and other non-coding RNAs, as well as their structures and functions.

Genomic Profiling of HCC

Comprehensive and integrative analyses on multiple data platforms in conjunction with clinical data allow us to paint a more detailed genomic picture of primary liver cancers (Figure 1). Genome-wide sequencing has revealed that HCC is similar to other common solid tumors in its frequency of genetic alterations, with **nonsynonymous somatic mutations** being identified in 30–50 genes per tumor on average (www.cbioportal.org/) [27–37]. However, other genomic, transcriptomic, and metabolomic aspects of hepatocarcinogenesis are unique. For example, compared to other cancers, HCC presents with divergent transcriptomic changes, unique global expression patterns, and significant differences in the expression of essential genes catalyzing tricarboxylic acid (TCA) cycle metabolism [38]. Consistent with many previous genomic analyses, **The Cancer Genome Atlas** (TCGA) network group demonstrated that *TERT* promoter mutations (44%), *TP53* tumor-suppressor mutations (31%), and *CTNNB1* oncogene mutations (27%) are the most common somatic genetic alterations in HCCs, suggesting that telomere maintenance, p53, and Wnt/β-catenin signaling pathways may play a common role in HCC pathogenesis [27]. Other somatic mutations that occur in up to 10% of HCC samples include the chromatin remodeling pathway (*BAP1*, *MLL*, *ARID1A*, *ARID2*, etc.), the RTK/KRS/PI3K pathway (*MET*, *FGFR1*, *VEGFA*, *KRAS*, *PIK3CA*, *PTEN*, *TSC1/2*, *RP6SKA3*, etc.), the cell-cycle pathway (*RB1*, *CDKN2A*, *CCNE1*, etc.), the oxidative stress pathway (*NFE2L2* and *KEAP1*), and the JAK–STAT pathway (*JAK1*, *IL6R*, *IL6ST*, etc.). We summarize the most common somatic mutations, chromosomal alterations, and non-coding RNA changes found in HCC and iCCA in Table 2 [39–43]. Several excellent reviews have detailed the genomic landscape in HCC and iCCA [2,5–7]. We highlight some of the most

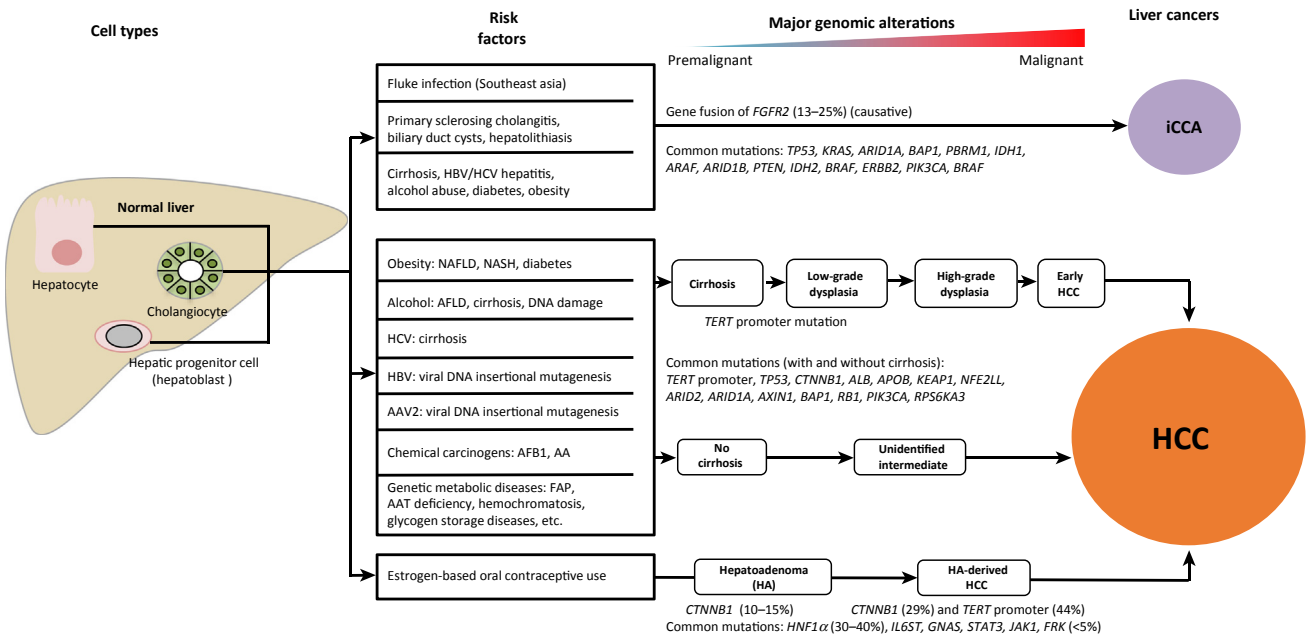

Figure 1. Genomic Alterations and Tumorigenesis in HCC and iCCA. HCC and iCCA derive from multiple distinct cellular origins. Development of malignancy entails a complex interaction of unique and overlapping risk factors, premalignant genomic alterations and malignant-transforming alterations. Abbreviations: AA, aristolochic acid; AAT, α1 antitrypsin; AAV2, adeno-associated virus type 2; AFB1, aflatoxin B1; AFLD, alcoholic fatty liver disease; FAP, familial adenomatous polyposis coli; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; iCCA, intrahepatic cholangiocarcinoma; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

Table 2. Genomic Profiling in HCC and iCCA[a]

Cancer types / Characteristics		HCC							iCCA			
Subtypes			Viral		Alcohol	Chemical carcinogens and others	Precancerous Lesions		Fluke infection	Non-fluke infection	HBsAg Seropositive	HBsAg Seronegative
		HBV	HCV	AAV2			Cirrhotic macronodules	HCA				
Somatic mutations	Significant mutations	TP53 (53.4%), HBV insertional mutations in TERT promoter, and MLL4, CCNE1	TERT promoter (62.9%)	AAV2 insertional mutations in TERT promoter, CCNA2, CCNE1, TNFSF10, MLL4	CTNNB1 (74%), CDKN2A (9%)	AFB1: R249S in TP53 AA: mutations with T>A transversion FAP: APC in germ-line hemochromatosis: HFE1(C282Y) glycogen storage diseases: G6PC/T	TERT promoter mutations in LGDN (6%) and HGDN (19%)	HNF1α mutation (30–40%) (germline HNF1A mutation is associated with MODY3)	TP53 (39–44%); TGF-β component SMAD4 (16–19%)	BAP1 (10–32%), IDH1/2 (9–10%)	TP53 (50%)	KRAS (28%)
	Common mutations (average > 10%)	TP53, CTNNB1, ALB, APOB, KEAP1, NFE2LL, ARID2, ARID1A, AXIN1					None	CTNNB1 (hot spots in exon 3), IL6ST	TP53, KRAS, ARID1A, BAP1, PBRM1, IDH1, ARAF			
	Less common mutations (average < 10%)	BAP1, SPTBN1, RB1, PIK3CA, RPS6KA3, AHCIT1, CDKN2A, CCNE1, FGF19, MLLs, TSC1/2, ATR, FANCD2, FANCM, FAN1, TP53BP1, BRCA1/2, JAK1, LAMA2, VCAM1, CDK14					None	GNAS, FRK, STAT3, JAK1	ARID1B, SMARCD1, STK11, PTEN, IDH2, BRAF, ERBB2, PIK3CA, BRAF, BRCA2, ATM, MLL3, APC, NF1, ELF3, ALB,FGFR2			
Chromosomal aberrations	Copy number gains: Chr 1q and 8q LOH: Chr 8p and 17p Deletion: CDKN2A, ERRFI1, RB1, NCOR1, PTEN Amplification: CCND1, FGF19, MYC, MET, VEGF, MCL, TERT					FAH deletion in hereditary tyrosinemia type I HMBS deletion in AIP Polymorphisms of HLA locus on chromosome 6p21.3 in AIH	N/A	Copy number gains: Chr 1q, 7 LOH: chr12q Deletion:TCF (HNF1)	Gene fusions: FGFR2 (13-25%), BICC1, ROS Amplification: CCND1, Myc, YEATS4, MDM2, EGFR Deletion: CDKN2A			
Transcriptome	HBV-insertion in genes leads to high RNA expression of MLL4, TERT, CCND1, CCNE1, GLI2					Increased SERPINA1 in AAT deficiency	Increased transcription of telomerase in TERT-mutated macronodules	Decreased LFABP. Increased GLUL, LGR5 Overexpression of ASS1 in bleeding HCA	Increased immune checkpoint genes CTLA4, ID01, HAVCR2, TNFRSF9, BTLA, CD274, PDCD1, LAG3			

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Table 2. (continued)

Cancer types / Characteristics	HCC			iCCA
Epigenetic modification	Hyper-methylation of the promoter region of silenced genes: CDKN2A (53%), HHIP, PTGR1, TMEM106A and CPS1	N/A	Hyper-methylation of the promoter region of silenced genes: SLC22A1, HABP2, LECT2 and ACSM3	Hyper-methylation of the promoter region of silenced genes: CDKN2A (21%), BAP1, PBRM1 and ARID1A
Non-coding RNA	TERT promoter mutations (40-60%) Mutations in lncRNA of NEAT1, MALAT1 Mutations in gene promoters of TFP12, MED16, WDR7 Mutations in UTRs of BCL6, AFF4 Mutations in CTCF-binding regions on Chr 2, 3, 18, and 20 Increased miR-221, miR-224, miR-21, and miR210 Decreased miR-122, miR-1, miR-124, miR-214, miR-34-A, miR-449			N/A
Altered pathways	Telomere maintenance Wnt/β-catenin P53/cell cycle Epigenetic remodeling Oxidative stress response TGF-β pathway DNA damage response PI3K/AS/RAF/MAPK pathway Metabolic reprogramming	Telomere maintenance	Glycolysis/ gluconeo-genesis Fatty acid synthesis Estrogen metabolism JAK–STAT Wnt/ β-catenin mTOR pathway	KRAS/RAF Epigenetic remodeling/SWI/SNF complex Wnt/β-catenin Cell cycle TGF-β pathway Metabolic/mitochondrial pathways
References	[27–46,48,53]			[73,105–116]

[a]Abbreviations: AIP, acute intermittent porphyria; HBsAg, hepatitis B surface antigen; HGDN, high-grade dysplastic nodule; LGDN, low-grade dysplastic nodule; lncRNA, long non-coding RNA; LOH, loss of heterozygosity; miR, microRNA; N/A, not applicable; UTR, untranslated region.

recent discoveries from liver cancer genomic profiling and address their therapeutic potential in cancer treatment below.

TERT Promoter Mutations and Liver Cirrhosis

TERT promoter mutations have been reproducibly associated with cirrhosis and cirrhosis-associated HCC [7]. *TERT* promoter mutations were analyzed by whole-exome sequencing of human tissue samples and were found in 6% of low-grade dysplastic nodules and 19% of high-grade dysplastic nodules in cirrhosis, as well as in 61% of early-stage HCC samples [29]. The frequency of these mutations remains stable in progressed and advanced HCC, demonstrating that a *TERT* promoter mutation is one of the earliest recurrent somatic genetic alterations during the transformation sequence from liver cirrhosis to HCC [44,45]. *TERT* promoter mutations occur during long-term malignant transformation, often in the context of cirrhosis, alcohol abuse, or metabolic syndrome, and are significantly more likely to be found in older, male, and HCV-associated HCC patients [27]. *TERT* promoter mutations (54–60%) [44], *TERT* amplification (5–10%) [27,30], and HBV insertion into the *TERT* promoter (10–15%) can all drive increased levels of telomerase expression [35,46]. Interestingly, *TERT* promoter mutation did not significantly correlate with increased *TERT* RNA expression, but significantly correlated with oncogene *CTNNB1* mutations in HCC patients [29,30,44] and epigenetic silencing of the tumor-suppressor gene *CDKN2A* (p16INK4A) [27], suggesting that activated telomerase may cooperate with other genes to initiate liver tumor formation. A recent study described the mutational landscape of HCC by analyzing liver cancer cases from different populations by whole-exome sequencing, and demonstrated that a hotspot *TERT* promoter mutation, *TERT* focal amplification, and/or viral genome integration occurs in >8% of cases, implicating *TERT* as a central and ancestry-independent feature underlying hepatocarcinogenesis [30]. Of human HCCs, 90% harbor increased telomerase expression, suggesting that HCC might potentially be targeted with telomerase inhibitors [47].

Tumor-Suppressor *TP53* Mutation and HBV-Associated HCC

The tumor-suppressor gene *TP53* is mutated in 20–52% of HCC patient samples [27–29,31–33,35–37,46,48]. Many *TP53* mutations have been identified [27], but few of these represent recurrent *TP53* mutation hotspots. One notable exception is the R249S mutation that is found in 10% of 115 AFB1-associated HCCs in the TCGA database [27,29]. *TP53* mutations but not *TERT* promoter mutations were significantly associated with HBV-related HCCs [27,29]. Moreover, HBV-positive HCC samples had much higher AFB1 activity than HBV-negative HCC, suggesting a synergistic interaction between AFB1 exposure and HBV infection, likely mediated through *TP53* mutations. The majority of HCCs with *TP53* mutations have elevated levels of p53 transcriptional targets, suggesting that mutant *TP53* not only causes loss of wild-type tumor-suppressing p53 functions but also **gain-of-function** [27]. *TP53* alterations lead to various hallmark cancer features, including genomic instability, anti-apoptotic activity, dysregulation of the cell cycle, and an impaired DNA damage response, all of which contribute to HCC tumorigenesis [27,49,50]. Therefore, understanding the activity and function of activated mutant *TP53* is a particular research priority because many of these mutations and pathways represent attractive and potentially druggable targets [51].

WNT/β-Catenin Signaling Pathway and the Malignant Transformation Process in HCC

The WNT/β-catenin signaling pathway plays a crucial role in embryogenesis, differentiation, and cell proliferation in the liver [52]. *CTNNB1*, the gene that codes for β-catenin, is the most oncogenic member of the WNT pathway and is frequently mutated (10–37%) in HCC patient samples [27,29,30,33]. In addition, inactivating mutations in *AXIN1* (3–16%) and *AXIN2* (3%), both negative regulators of the Wnt pathway, as well as rare inactivating mutations of the tumor-suppressor gene

adenomatous polyposis coli (*APC*), have been reported to contribute to Wnt pathway activation with subsequent tumorigenesis [34]. HCCs with activating *CTNNB1* mutations are usually subclassified within the nonproliferation subgroup of HCC, and typically constitute less-aggressive, well-differentiated tumors with low levels of α-fetoprotein compared to tumors from the proliferation subgroup [7]. *CTNNB1* mutations are rarely found in conjunction with mutations of *TP53*, which is strongly correlated with HBV infection. However, *CTNNB1* mutations are associated with *TERT* promoter mutation, an alteration predominantly associated with HCV-related cirrhosis and HCC [44,45].

CTNNB1 mutations typically occur following *TERT* promoter mutations in the development of cirrhosis, suggesting cooperation between telomerase maintenance and the β-catenin pathway and its precursors in patients with HCC [7]. *CTNNB1* was also frequently mutated (29%) in patients with HCA-transformed HCC in the absence of cirrhosis [8]. Furthermore, *CTNNB1* mutations are the most frequently reported molecular event (70%) in hepatoblastoma [53], suggesting that an activated oncogenic WNT/β-catenin signaling pathway is associated with liver cancer initiation and progression regardless of cirrhotic status. The frequent genetic alteration of WNT/β-catenin signaling found in HCC highlights the appeal of therapies designed to selectively target of this pathway. However, because WNT/β-catenin signaling often cooperates with other signaling pathways and is highly context-dependent, further research is clearly necessary to further explore the potential value of combinatorial therapies [54,55].

Distinct Context-Dependent Roles of the TGF-β Signaling Pathway in HCC

Genomic alterations in the TGF-β signaling pathway have been reported in 87% of the hypermutated colorectal cancers with microsatellite instability [56], and alterations of several TGF-β members and their target genes are also seen in liver cancers [57–60]. Analysis of somatic mutation data from a cohort of 202 TCGA HCC samples showed that 38% overall had somatic mutations in TGF-β pathway genes [59]. *SPTBN1*, encoding a key adaptor for SMAD3 nuclear translocation, was the single most mutated TGF-β pathway gene (6%) in HCC, indicating its important role in many cases of TGF-β-associated HCC tumorigenesis and progression [59–61]. The highest incidence of somatic mutations in the DNA damage repair pathway in HCC patient samples was found in the **Fanconi anemia genes** *FANCM* (9%) and *FANCD2* (10%), key genes involved in the interstrand crosslink DNA repair pathway [62].

Similarly to *TP53* mutations in HCC, mutations of both TGF-β pathway genes and DNA repair genes do not result in inhibition of mRNA expression, and gene products with complex potentially oncogenic functions may be expressed [59]. Importantly, mutations in several of the DNA repair pathway genes such as *ATR*, *FANCD2*, and *TP53BP1* were found to be significantly associated with mutations in TGF-β pathway genes in HCC patient samples [59]. Both TGF-β signaling and functional Fanconi anemia genes are required to maintain genomic stability and prevent DNA damage, such as interstrand cross links, in the presence of environmental toxins such as alcohol [62–64], suggesting one possible source of a cooperative oncogenic effect between these two pathways.

TGF-β is also an immunosuppressive cytokine produced by liver tumor cells and its surrounding immune cells such as T cells and macrophages [65]. TGF-β enhances antigen-induced PD-1 expression and mediates T cell suppression in a SMAD3-dependent manner [65]. A recent study established **immune classes** within HCC, including exhausted and active immune subclasses [66]. TGF-β signatures, such as WNT/TGF-β [67] and **late TGF-β signatures** [68], are clearly enriched in the exhausted immune class of HCC, a subtype with a particularly poor prognosis [66].

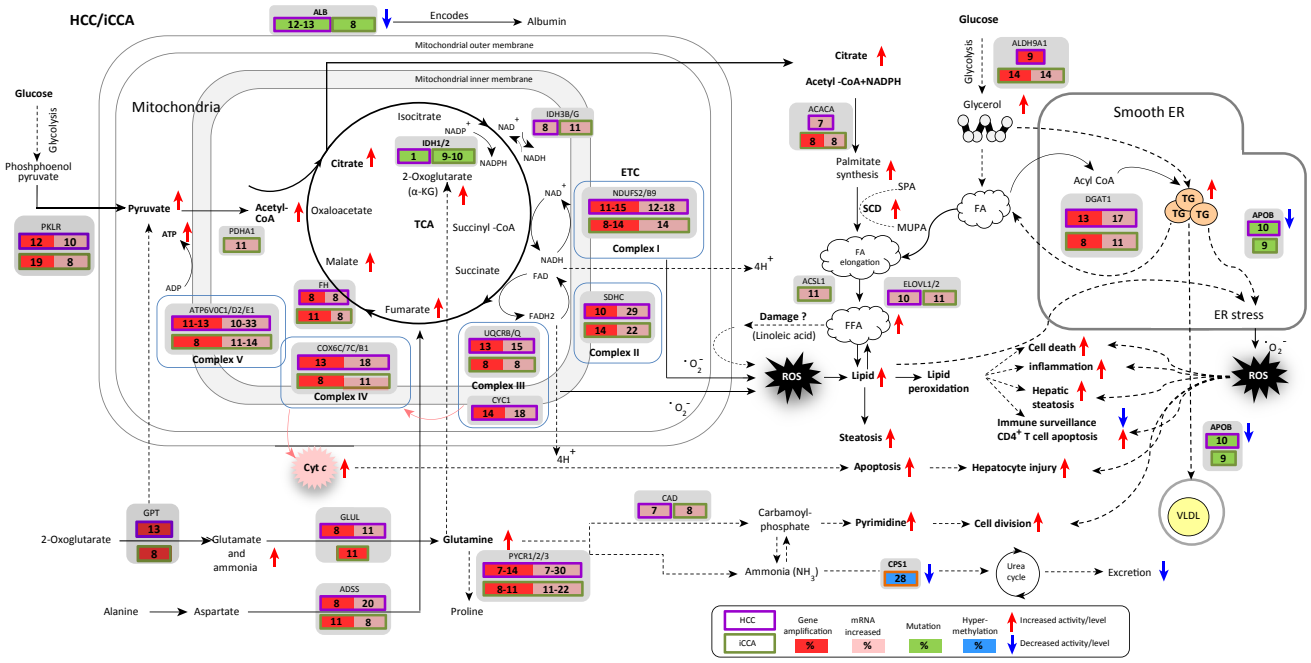
While TGF-β signatures have been predominantly demonstrated in tumor promotion, comprehensive integrated analyses for TGF-β superfamily genes using the TCGA HCC cohort identified a subset of tumors in which TGF-β serves as a tumor suppressor [59]. Two TGF-β signatures were identified, named activated and inactivated, reflecting the dual nature of TGF-β signaling in liver cancer. Consistent with previous studies, the majority of HCCs (60%) in the TCGA cohort demonstrated an 'activated' profile of TGF-β, characterized by an activation of diverse oncogenic pathways such as the KRAS proto-oncogene, MDM2 proto-oncogene, mechanistic target of rapamycin kinase (MTOR), insulin-like growth factor 2 (IGF2), and vascular endothelial growth factor A (VEGFA), as well as stimulation of the immune response [69]. These activated HCCs also expressed increased markers of liver inflammation, fibrosis, and cirrhosis [69]. By contrast, a smaller share of tumor samples (20%) demonstrated inactivated TGF-β and loss of tumor-suppressor function, with a genomic profile characterized by decreased DNA repair activity. In these analyses, both the activated and inactivated TGF-β signature leads to a poor overall survival compared to the remaining ~20% of tumors that have TGF-β pathway activity close to that of normal livers [38].

These TGF-β signatures were also validated in different independent cohorts. Interestingly, the subgroup of patients with the inactivated TGF-β signature had an even worse survival rate than patients with the activated TGF-β signature, suggesting that potential therapies designed to block TGF-β signaling should only be considered in patients with an activated TGF-β HCC signature, and may carry substantial risk otherwise [59]. The signatures also provide a logical approach toward targeting HCC. For instance, targeting VEGF (either directly by VEGF inhibitors or indirectly by kinase inhibitors such as sorafenib) may prove to be efficacious only in HCC with an activated TGF-β signature or *VEGF* amplification [70]; by contrast, targeting FGFs may prove to be important in HCC with an inactivated signature. This putative link between the TGF-β pathway, the proinflammatory immune/tumor microenvironment, and DNA repair suggests a context-dependent role of TGF-β signaling in inflammation, cancer immunity, and genomic integrity. Current evidence suggests that targeting a signaling pathway in cancer will be challenging and that therapies targeting the TGF-β pathway in HCC should only be considered in specific subtypes. Potentially, future biomarker-driven targeted therapeutic approaches to HCC could combine multiple signatures to find optimal agents specific to each HCC patient and alter the course of this lethal cancer [59].

Metabolic Homeostasis in Primary Liver Cancers

Metabolic Reprogramming in HCC and iCCA

Recently, obesity, diabetes, and fatty liver disease, recognized as key components of metabolic syndrome, have been identified as risk factors for HCC and iCCA [1,13,71,72]. Accumulating evidence suggests that alterations of metabolic reprogramming in liver cancer cells affect insulin uptake and glucose use, leading to an enhanced capacity for cell proliferation and nutrient use [27,72,73]. *ALB*, encoding a key mediator of hepatocyte function in the secretion of albumin in the blood, was frequently found to be mutated in HCC (12–13%) and iCCA (8%) [27–29,73]. APOB, a major protein constituent of chylomicrons, LDL, and VLDL that mediate fat and vitamin absorption and digestion in liver, was also frequently mutated in HCC (10%) and iCCA (9%) [27–29,73]. The TCGA network group reported decreased mRNA expression of *ALB* and *APOB* in HCC [27]. Similarly, *IDH1* and *2*, encoding the isocitrate dehydrogenases in the citrate cycle in mitochondria, were frequently mutated in iCCA (9–10% of cases), and in four HCCs with a poor prognosis in the TCGA dataset [27,73]. IDH-mutant iCCAs display high mitochondrial gene expression [73]. These findings raise the hypothesis that malignant liver cells might support their growth by reducing energy spent on non-essential metabolic activities [27].



Trends in Molecular Medicine

Figure 2. Metabolic Reprogramming in HCC and iCCA. Genomic alterations in HCC and iCCA have the potential to alter multiple metabolic processes including glycolysis, FA synthesis, glycerolipid metabolism, alanine/aspartate/glutamate metabolism, and the tricarboxylic acid (TCA) cycle/electron transport chain (ETC) in mitochondria. The frequency (%) of gene expression changes, gain of gene copy number, mutations, or hypermethylation in HCC or iCCA based on the TCGA dataset (Cancer Genome Atlas Research Network; www.cbioportal.org/www.cbioportal.org/ [27,73]) was noted for each gene. The purple and green boxes represent HCC or iCCA samples, respectively. Decreased or increased activity of each gene in the context of liver cancer (either HCC or iCCA) is noted with red or blue arrows, respectively. Abbreviations: Cyt, cytochrome; ER, endoplasmic reticulum; FA, fatty acid; FFA, free fatty acid; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; ROS, reactive oxygen species; TG, triacylglycerol.

We therefore investigated the genomic alterations related to metabolic processes in HCC and iCCA using TCGA datasets (www.cbioportal.org/) [74–76]. These processes include glycolysis, FA synthesis, glycerolipid metabolism, citric acid cycle/electron transport reaction in mitochondria (TCA cycle/ETC), and alanine/aspartate/glutamate metabolism. Although few mutations have been documented in these pathways in HCC or iCCA, gain of copy number and/or increased expression have been identified in a majority of metabolism-associated genes (Figure 2) [27,38].

Metabolic changes may also lead to FA and lipid accumulation, both promoting development of a fatty liver and supporting cancer metabolic needs. Recently, Budhu and colleagues performed gene expression as well as metabolic profiling of HCC from clinical samples, and identified a lipogenic network that involves elevated concentrations of lipid metabolites of stearoyl-CoA desaturase (SCD) activity and palmitate signaling (Figure 2) [77]. Increased SCD, an enzyme responsible for the conversion of saturated palmitic acid (SPA) to monounsaturated palmitoleic acid (MUPA), was significantly associated with HCC progression and poor patient outcomes, suggesting that SCD activity in tumor cells may play a significant role in facilitating aggressive HCC by regulating the balance between SPA and MUPA (Figure 2) [77]. Consistent with these data, the TCGA database for HCC and iCCA indicates that increased expression of metabolic genes (involved in FA biosynthesis and glycerolipid metabolism) may lead to glycerol accumulation and indirectly increase the production of FA and triacylglycerol (TG). These genomic alterations may rebalance the distribution of energy in liver cells, leading to fatty liver,

one of the most common risk factors for liver fibrosis, cirrhosis, and malignant transformation in both HCC and iCCA, and providing a source of energy that feeds cancers [27] (Figure 2).

In addition, lipid accumulation can result in endoplasmic reticulum (ER) stress, which is characterized by an altered composition of lipids in the ER and inhibition of the hepatic ER calcium ATPase [78]. ER stress leads to oxidative stress that promotes necrotic cell death, liver damage, inflammation, hepatic steatosis, and proliferation [79,80], all of which have been documented to promote malignant transformation (Figure 2). Recently, Ma and colleagues reported that metabolic dysregulation of lipids in hepatocytes decreases hepatic immune surveillance and promotes HCC tumorigenesis by reactive oxygen species (ROS)-mediated depletion of $CD4^+$ T cells (Figure 2) [81]. NAFLD-associated lipid disruption, characterized by increased levels of FA (linoleic acid), upregulates mitochondrial ROS and superoxide production in murine hepatic $CD4^+$ T cells, promoting apoptosis. However, the detailed mechanisms by which FA (linoleic acid) induces mitochondrial dysfunction in $CD4^+$ T cells are unclear.

Several enzymes that regulate the levels of glutamine, proline, and aspartate are essential for providing precursors to the TCA cycle and amino acid biosynthesis, and were found to be upregulated in HCC and iCCA in the TCGA database (Figure 2) (www.cbioportal.org/). *CPS1* encodes a liver-specific rate-limiting enzyme for the excretion of ammonia generated by glutamine via the urea cycle (Figure 2). Frequent hypermethylation of *CPS1* was found in HCC (28%), and hypermethylation-mediated downregulation of *CPS1* led to decreased usage of glutamine in liver cancers [27]. Increased *CAD* (carbamoyl phosphate synthase II) catalyzes glutamine conversion to carbamoyl-phosphate and initiates the *de novo* pyrimidine synthesis pathway, thus favoring cell division and tumor proliferation [27].

Obesity-Associated Dysmetabolism in Liver Cancers

Non-alcoholic fatty liver disease (NAFLD) is becoming increasingly recognized as an important contributor to the burden of HCC and iCCA worldwide [71,73,82]. HCC and iCCA development in NAFLD is multifactorial and complex, involving chronic inflammation, tissue damage, regeneration, remodeling, and uncontrolled proliferation, finally leading to carcinogenesis. Obesity and dysmetabolism serve as drivers of oncogenesis in the setting of abnormal hepatic morphology, and hepatic steatosis may provide the appropriate microenvironment for the development of liver cancers (Figure 3) [80,83].

The expansion and remodeling of adipose tissue in obesity leads to massive release of several proinflammatory cytokines, including TGF-α, IL-6, IL-1β, IL-17A, and TGF-β. These cytokines promote malignant progression through activation of NF-κB and STAT3 in hepatocytes in mouse HCC models [79,84,85]. High leptin and low adiponectin levels are two hallmarks of obesity and are both involved in NAFLD progression and carcinogenesis through the JAK/STAT3 and PI3K/Akt/mTOR signaling pathways in both human HCC and mouse HCC models [86,87]. Obesity leads to the development of both hepatic and systemic insulin resistance, and is worsened by hepatic lipid accumulation [88]. The lipotoxicity from *de novo* lipogenesis, which is regulated by mTOR-mediated release of SREBP1, leads to mitochondrial dysfunction and causes hepatic damage via ROS generation [89]. In addition, TG accumulates in hepatocytes and causes hepatic steatosis, sensitizing hepatocytes to TNF-induced inflammation after its accumulation in mitochondria [90].

Dysfunctional adipose is associated with increased infiltration of various types of immune cells from both the innate and adaptive immune systems. $CD8^+$ T cells and natural killer T (NKT) cells contribute to NASH development and hepatic malignant progression through the LTβR ligand

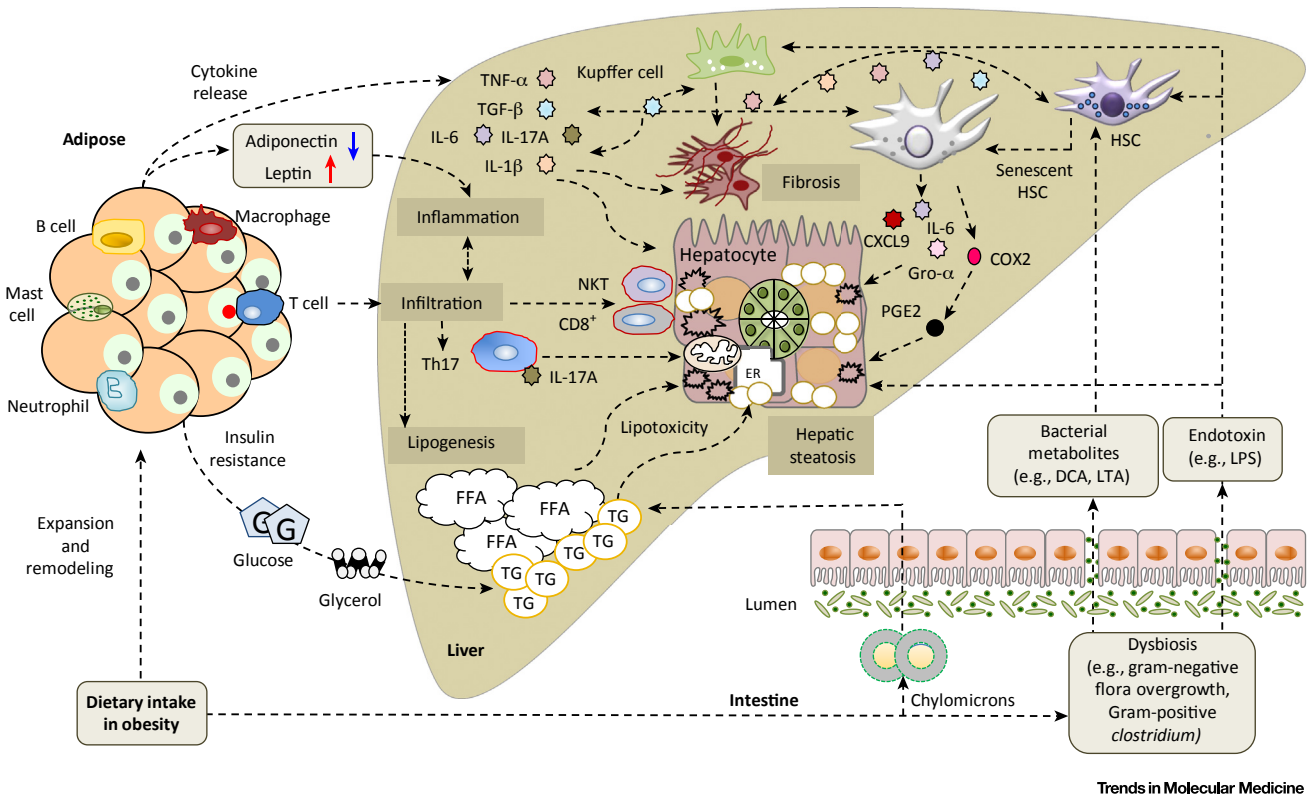


Figure 3. Mechanisms of Obesity-Associated Dysmetabolism in Liver Cancers. The expansion and remodeling of adipose tissue in obesity promotes liver inflammation, lipogenesis, hepatic steatosis, fibrogenesis, and insulin resistance by the release of proinflammatory cytokines and adipokines, immune cell infiltration, and hyperinsulinemia. These changes activate Kupffer cells, hepatic stellate cells (HSCs), and senescent HSCs, and damage hepatocytes and cholangiocytes. Obesity-associated gut dysbiosis and increased gut permeability lead to release of gut-derived microbiota-associated endotoxin and bacterial metabolites, such as lipopolysaccharide (LPS), deoxycholic acid (DCA), and lipoteichoic acid (LTA), which in turn promote the senescence-associated secretory phenotype (SASP), hepatic inflammation, and fibrosis to drive liver cancer pathogenesis. Abbreviations: $CD8^+$, cytotoxic T cell; COX2, cytochrome *c* oxidase subunit 2; CXCL9, chemokine (C-X-C motif) ligand 9; ER, endoplasmic reticulum; FFA, free fatty acid; Gro-α, chemokine (C-X-C motif) ligand 1; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-17A, interleukin 17A; NKT, natural killer T cell; PGE2, prostaglandin E2; TG, triacylglycerol; TGF-β, transforming growth factor β; Th17, type 17 T helper cell; TNF-α, tumor necrosis factor α.

LIGH and NF-κB signaling pathways based on a mouse model of dysmetabolism in which animals are chronically fed a choline-deficient high-fat diet [91]. Excess nutrients led to unconventional prefoldin RPB5 interactor-dependent DNA damage in hepatocytes in mice fed a high-fat diet, and this triggered inflammation via type 17 T helper (Th17) cells and IL-17A [85]. In addition, NAFLD-induced lipid accumulation and elevated FA (linoleic acid) cause selective $CD4^+$ T lymphocyte loss and promotes hepatocarcinogenesis, suggesting a crucial role for the adaptive immune response in NAFLD-driven HCC tumorigenesis [81].

NAFLD has been shown to be linked to small intestinal bacterial microbiome (**dysbiosis**) and increased intestinal permeability (Figure 3) [92–94]. Gram-negative bacteria (flora) produce lipopolysaccharide (LPS) which accelerates liver fibrogenesis, introducing dysbiosis as a potential cofactor contributing to chronic liver injury in NAFLD [94]. Deoxycholic acid (DCA), a secondary bile acid produced mainly by the Gram-positive bacterium *Clostridium*, can produce ROS leading to DNA damage and release of IL-6, Gro-α, and CXCL9 from senescent hepatic stellate cells (HSCs), thus facilitating HCC development in obese mice [95]. Obesity-induced lipoteichoic acid (LTA), a Gram-positive gut microbial component, promotes HSC

release of COX2 and PGE2, creating a tumor-promoting microenvironment [96]. These models provide putative evidence of the link between gut dysbiosis and hepatocarcinogenesis in NAFLD and obesity [92]. Obesity and its sequelae such as insulin resistance, adipose remodeling, and alterations in the gut microbiota are important intermediaries in the initiation and propagation of oncogenesis in NAFLD.

Most recently, data from three independent groups demonstrated that gut microbiota modulate response to anti-PD-1 immunotherapy in melanoma patients, suggesting that the microbiome may determine patient suitability for cancer immunotherapies [97–99]. In summary, obesity-induced alterations of the immune- and gut microbiota-mediated liver cancer microenvironment may be key determinants of liver cancer growth and response to extrinsic signals. In the future, liver cancer therapies might target an abnormal microbiome or be directed only at patients with a specific signature microbiome.

Concluding Remarks

Primary liver cancers represent a diverse set of cancers with mixed risk factors and a frequently multistep pathogenesis [3,5,6]. Although genetic and genome-wide studies have provided a big picture of the main oncogenic drivers and pathways in HCC and iCCA, the detailed mechanisms in the initiation and progression of these diseases are still lacking, in part because the long interval between acquisition of the earliest mutations in precancerous disease and active malignancy entails alterations in numerous tumor-promoting factors and pathways. The central role of *TERT* promoter mutations in cirrhosis and cirrhosis-associated liver carcinogenesis has been well established [7]. Accumulating epidemiological and pathological evidence also demonstrate that HCC may occur without cirrhosis [7,10,11]. The mechanism and genomic alterations in non-cirrhotic HCC have not yet been identified (see Outstanding Questions and Box 2).

Box 2. Clinician's Corner

HCC and iCCA are typically diagnosed at a late stage, in part owing to the lack of effective and accurate biomarkers for early detection. Genomics and gene signatures have the potential to address this substantial unmet clinical demand for effective screening and early diagnosis.

Currently, ultrasonography is the recommended modality for HCC screening and surveillance according to the EASL/EORTC (European Association for the Study of the Liver/European Organisation for the Research and Treatment of Cancer) guidelines.

Liver tissue biopsy is limited in use for HCC and iCCA diagnosis owing to challenges of tissue sampling as well as procedural risks. Development of novel testing for genomic analysis in combination with ultrasonography may improve strategies for liver cancer prevention and surveillance.

Treatment of liver cancers is beginning to incorporate findings from large genomic studies. As these technologies gain greater acceptance in clinical use, molecular signatures will become increasingly central to the delivery of personalized targeted therapy for liver cancers.

The failure of many clinical trials for HCC and iCCA to deliver new effective treatments may be due an incomplete understanding of the underlying molecular pathogenesis. Translating our knowledge of the landscape of mutations, genomic alterations, and diagnostic/prognostic signatures occurring in primary liver cancer may not only help clinical management but will also help to develop more effectively designed clinical trials.

Obesity or NAFLD/NASH-induced chronic liver inflammation and fibrosis alter the liver microenvironment and are recognized as the major proximate risk factors for development of HCC and iCCA. This common pathway represents an attractive potential target for an immune-based therapeutic approach. Potent liver-directed antifibrotic therapies may also contribute to HCC and iCCA risk-reduction strategies.

Outstanding Questions

How can genome-wide analyses be used to evaluate and/or select therapeutics (e.g., immunotherapies, combinations of molecular targeted therapies) in liver cancer patients?

Do primary liver cancer subtypes share common mechanisms of tumor initiation, hepatocyte/cholangiocyte/hepatoblast differentiation, and malignant transformation?

What genomic and epigenomic alterations occur following liver injury or damage (e.g., mitochondrial dysfunction, ER damage, defective macroautophagy, liver immune cell infiltration)? Can mechanistic investigation of these changes aid in the development of new strategies to treat liver cancers?

Can we develop an alternative biopsy for liver cancers (e.g., genome-wide analysis of circulating liver cancer cells or cell-free circulating liver tumor-specific DNA)?

How does the increasing rate of obesity and NAFLD/NASH contribute to liver cancer disease burden? What molecular pathways distinguish NAFLD/NASH-associated liver cancers from liver cancers of other causes? How do these obesity-related risk factors interact with known risk factors such as hepatitis?

How can genomic databases across different countries, populations, and study groups be combined to guide clinical practice at a molecular level? How can the complex set of potentially overlapping cancer subclassifications from genomic studies be simplified?

The relative complexity of genomic alterations in HCC and iCCA currently confounds the translation of basic research findings into clinical practice [2,3]. Furthermore, current diagnostic approaches, even when using tissue biopsy, may not accurately reflect the collection of molecular changes driving liver pathology. For example, a single liver biopsy from a multifocal nodular cirrhotic liver is unlikely to capture the genetic diversity found in the liver overall [100,101]. Moreover, sampling errors and hemorrhage can occur following needle biopsies, and needle-track tumor seeding has been reported after sampling of malignant lesions [102]. Despite the power of genome-wide studies, these practical limitations need to be resolved before genomic profiling can be fully trusted and adopted. While unable to characterize liver histologic architecture, future technologies using noninvasive liver-specific liquid biopsy (i.e., from peripheral blood) have the potential to address many of these limitations [103].

There are still no effective curative therapeutics for advanced-stage HCC and iCCA, the two major primary liver cancers and the types with highest mortality [3,4]. No new drugs have shown positive results in clinical trials since the identification of sorafenib in HCC, although nivolumab is a promising new adjuvant therapy. Many overlapping genomic, transcriptomic, and metabolomic alterations have been found in HCC or iCCA patient samples, including alterations in TP53/cell cycle, the TGF-β pathway, epigenetic/chromosome remodeling, Wnt/β-catenin, and metabolic reprogramming pathways, implying that these altered genes or pathways may cooperate or compensate for each other in liver cancer pathogenesis [16,27,73]. Therefore, targeting one oncogene or pathway may not be sufficient to halt the uncontrolled growth of primary liver cancers. Future therapies may be focused on treatment of the downstream effectors of these identified molecular pathways or may target a combination of particular pathways with sets of inhibitors (precision therapy or cocktail therapy).

In addition, while hepatocytes, cholangiocytes or hepatic progenitors are recognized as the primary cellular source of liver cancers, these cells do not exist in isolation. Their interactions with surrounding immune cells, sinusoidal endothelial cells, hepatic stellate cells, or Kupffer cells contribute to the set of signals driving hepatocytes towards malignant transformation [104]. Obesity-induced alterations of the immune system and gut microbiota mediate the liver cancer microenvironment and may represent modifiable disease pathways [80,83,92]. Future drugs that act on the liver microenvironment have the potential to prevent malignant transformation or restrain growth of already-established malignancies. To date, none of these additional pathways has been targeted in clinical applications.

Liver cancer fundamentally represents unrestrained clonal proliferation of abnormal liver cells whose oncogenic properties stem from a series of distinct molecular alterations. Therefore, a systematic characterization of these alterations at the genomic level and an understanding of how their effects can be mitigated represent one of the most compelling approaches to fighting this disease. In the near future we anticipate identification of additional tumor-specific cancer genomic and molecular features. It is highly likely that many of these will be used to provide individualized therapeutic options for patients with primary liver cancers and ultimately benefit patient survival.

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