Review

Hepatocellular carcinoma pathogenesis

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Cross talk between genetics and biochemistry in the pathogenesis of hepatocellular carcinoma

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Abstract

The liver is a crucial organ in the regulation of metabolism, signaling, and homeostasis. Using recent advanced sequencing technologies, several mutations of genes in major metabolic and signaling pathways have been discovered in the pathogenesis of hepatocellular carcinoma (HCC). These gene signatures alter expression and ultimately affect biochemical pathways by modifying enzyme/protein levels, resulting in numerous clinical outcomes related to HCC. It comes with varying forms of genetic and biochemical alterations, associated with carbohydrate, lipid, nucleic acid, and amino acid metabolism, as well as signaling pathways linked to tumorigenesis. Here, we aim to summarize the main components and mechanisms involved in the progression of HCC with a special focus on the metabolic regulation of key effectors of tumorigenesis, through the crosstalk between genetics and biochemistry. This paper provides an overview of hepatocellular carcinoma, underlying the fundamental effect of gene variations on metabolic and signaling pathways. Since there is still an unmet need for biomarkers and novel therapeutic targets, some of these signature genes or proteins can be used as novel biomarkers for diagnosis, prognosis, and novel potential therapeutic targets for the treatment of HCC.

Keywords: Biochemistry; biomarkers; genetics; hepatocellular carcinoma; therapeutic targets; pathogenesis.

Introduction

Most liver diseases, characterized by an extremely dysregulated metabolism in hepatocytes, are characterized by low overall survival and poor prognosis. They have complex biological processes on gene and protein levels in their pathogenesis. As the liver is the main site of numerous metabolic events in energy metabolism by the regulation of several biomolecules, including carbohydrates, lipids, nucleic acids, and amino acids, the synergy of interconnected pathways should be assessed rather than isolated mechanisms in these diseases. HCC predominantly shows the Warburg effect. In addition, several pathways of lipid metabolism are dysregulated, such as fatty acid synthesis, β-oxidation, and cellular lipidic composition, to support HCC tumorigenesis. Increased aspartate, glutamine, and hydroxyproline correlated with the tumor burden. Altered miRNA expression, DNA copy number variations (CNVs), deletions, and insertions are prevalent genetic occurrences in HCC.[1] Identification of pre-cirrhotic patients who have the potential to progress to HCC is still an unmet dilemma in clinical practice. Novel biomarkers for diagnosis and/or prognosis and novel potential therapeutic targets for treatment are urgently needed. This paper provides an overview of HCC, highlighting the critical effect of gene variations on metabolic and signaling pathways, and their crosstalk among each other in the pathogenesis of liver diseases.

Hepatocarcinogenesis

Our understanding of the molecular pathogenesis of hepatocarcinogenesis has improved due to recent developments in Next Generation Sequencing (NGS) (Whole genome or exome sequencing) and Genome-Wide Association Study (GWAS) technologies. The molecular classification of HCC has so far been based on the genomic, transcriptomic, metabolic, and/or epigenomic profiling of large tumor cohorts employed in multi-omics research.[1] Studies using NGS have shown 40–60 somatic coding alterations per HCC, with 4–6 of them being driver mutations.[2] There are two mutation subtypes: proliferation and nonproliferation.[3] The proliferation class is defined by cell proliferation and survival pathways, such as PI3K-AKT-mTOR, RAS-MAPK, and MET, as well as chromosomal instability, TP53 inactivation, FGF19, and CCND1 amplifications.[4] This proliferation class is associated with HBV infection and α-fetoprotein (AFP) overexpression.[5] Nonproliferation classes often have activation of WNT signaling transducer β-catenin (CTNNB1) and telomerase reverse transcriptase (TERT) promoter mutations. These tumors are transcriptionally identical to normal AFP and are associated with alcohol use and HCV infection etiologies.[6]

Genetic Pathophysiology of HCC

The most common HCC mutation affects the promoter of the enzyme TERT.[7] Up to 60% of HCC patients carry TERT promoter mutations.[8] According to the underlying liver disease, the incidence of TERT promoter mutations varied greatly, with the greatest frequency in HCV-related HCC (44%), followed by non-viral (38%) and HBV-related HCC (23%).[9]

Telomerase is a cellular ribonucleoprotein enzyme. It increases cell proliferation through telomere elongation. TERT is a telomerase component responsible for the enzyme's catalytic activity. Its expression is typically

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strictly restricted in most normal cells but upregulated in several malignancies.[10] TERT expression is both transcriptionally and post-translationally controlled. Some cancer-associated TERT promoter mutations work by producing de novo transcription-factor-binding sites at the transcription level. The two most common TERT promoter mutations are 124C/T and 146C/T, often known as C228T and C250T, respectively. These two mutations produce putative ETS transcription factor-binding sites (TTCC), triggering TERT transcription. TERT promoter mutations are one of the mutually exclusive telomerase reactivations.[11] Higher TERT expression in advanced HCC might be attributable to causes independent from TERT promoter mutations, such as HBV integration into the TERT sequence, TERT gene amplification, or the accumulation of oncogenic pathways with tumor progression.[7] TERT promoter mutations are commonly linked to CTNNB1 mutations, indicating that the β-catenin pathway and telomerase maintenance are both involved in the development of liver tumors.[12] Novel copper complex (NCB) regulates methionine cycle-induced TERT hypomethylation to promote HCC cell differentiation.[13] The importance of alternate routes leading to telomere synthesis, such as alternative telomere lengthening, must be properly assessed.[14]

In the combined novel meta-analysis, the new TERT locus rs2242652(A) showed lower HCC risk. After controlling for sex, age, BMI, and type 2 diabetes, this protective link remained significant. Leukocyte telomere lengths were increased with TERT rs2242652(A).[9] Significant correlations between alcohol-related HCC and PNPLA3 and TM6SF2 polymorphisms are shown. They also observed a significant connection with rs708113 in the WNT3A-WNT9A region on chromosome 1q42, which lowered alcohol-related HCC risk. This variation improved immune cell infiltration of tumor tissues and decreased CTNNB1, which often precedes HCC. rs708113 did not prevent HCC patients with persistent HCV or NAFLD.[15,16]

The WNT/β-catenin signaling system is essential for embryogenesis, zonation, and metabolic regulation in the liver. The cytoplasmic β-catenin destruction complex regulates β-catenin stability. Composed of Axin, APC, CK1, and GSK3, the β-catenin destruction complex is responsible for phosphorylating the N-terminal serine and threonine residues of β-catenin. Following recognition by β-TrCP, phosphorylated β-catenin is ubiquitinated and destroyed through the proteasome pathway. After the Wnt ligand binds to the cell membrane receptors LRP5/6 and Frizzled, the C-terminus of Frizzled undergoes a conformational shift, resulting in the recruitment of Disheveled proteins (DVL). Then, DVL joins Axin through their shared DIX domain, resulting in the dissolution of the β-catenin destruction complex. Thus, β-catenin accumulates in the cytoplasm and is translocated into the nucleus, where it activates the transcription of downstream genes.[17] It is the oncogenic pathway most triggered in HCC by CTNNB1 activating mutations (11–37%) and AXIN1 inactivating mutations (5–15%) or adenomatous polyposis coli gene (APC) (1–2%).[5] CTNNB1 mutations, which code for β-catenin, are in-frame deletions or substitutions at a hotspot in the domain targeted by the APC/AXIN1/GSK3B inhibitory complex. Tumors with CTNNB1 mutations show a distinct transcriptome profile with upregulation of conventional target genes, such as glutamate ammonia ligase (GLUL) and leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), as well as a distinct histologic pattern with intratumor cholestasis.[18]

Numerous nongenetic pathways that activate Wnt/β-catenin have also been found in studies. These include overexpression of the Frizzled membrane receptor (FZD) and Wnt ligands; deregulated expression of microRNAs and long non-coding RNAs that regulate Wnt/β-catenin signaling; and promoter hypermethylation and associated silencing of the secreted Frizzled related protein 1 (SFRP1) gene, a Wnt/β-catenin antagonist.[19–21] In recent years, it has been shown that oncogenic or tumor suppressor noncoding RNAs control the canonical Wnt/β-catenin signaling pathway's major regulatory elements. This mechanism controls HCC cell proliferation, invasion, metastasis, and drug sensitivity. miR-1246 has been identified as a possible factor that promotes HCC tumor growth by inhibiting the expression of its target ROR. Notably, they found that artificial stimulation of miR-1246 expression or ROR knockdown significantly increases the metastatic ability of HCC in vitro and in vivo via activation of the Wnt/β-catenin pathway and promotion of EMT.[22] Similarly, USP22, a histone-modifying enzyme primarily regulated by miR-329-3p (normally downregulated in HCC), is associated with distant metastasis, poor prognosis, and high recurrence rates in HCC. Because of this, it critically modulates tumor cell proliferation, metastasis, DNA repair, and stemness via modulating Wnt/β-catenin pathways.[23]

Recently, it was discovered that miR-19a-3p and miR376c-3p may promote the Wnt/β-catenin pathway in HCC cells by targeting SOX6. Furthermore, they discovered that SOX6 may bind to β-catenin, preventing it from dissociating from the transcriptional complex and being translocated to the nucleus. Overall, the results indicated that both miR-19a-3p and miR-376c-3p are significantly expressed in HCC cells. This may play a role in HCC development by targeting SOX6 and disrupting the Wnt/β-catenin signaling pathway.[24]

At least half of HCC patients with frequent TP53 mutations (12–48%), the tumor suppressor gene more often mutated in cancer, had altered P53 cell cycle pathways. There has not been discovered another recurrent TP53 mutation hotspot other than the R249S mutation linked to AFB1 exposure.[25] The homozygous deletion of CDKN2A (2–12%) or RB1 mutations (3–8%) in HCC mostly inactivates the retinoblastoma pathway, which controls progression from the G1 to S phase of the cell cycle. Interestingly, tumors with poor prognosis have higher levels of genetic changes in CDKN2A and RB1, pointing to a role for P21 pathway inactivation in tumor aggressiveness.[14]

Receptor tyrosine kinase (RAS-RAF-MAPK) and phosphatidylinositol-3-kinase, Protein kinase B, and mammalian target of rapamycin (PI3K-AKT-mTOR) pathways are generally activated in HCC, due to amplification of areas including FGF19 (5%) and mutations in RPS6KA3 and RSK2 (5-9%).[26] Furthermore, in a subset of HCC, activating mutations of PIK3CA (0–2%) and inactivating mutations of TSC1 or TSC2 (3–8%) result in the activation of the AKT/MTOR signaling. Additionally, homozygous deletion of the tumor suppressor phosphatase and tensin homolog (PTEN), which is a PI3K kinase inhibitor, has been found in 1–3% of HCC.[12,14] An additional path of AKT/mTOR activation has been proposed: direct upstream activation via the insulin growth factor pathway. Activating mutations in RAS family genes are uncommon in HCC (2%), and inactivating variants in RP6SKA3, which codes for the RAS inhibitor RSK2, were found in (2–9%) of tumors.[26] RSK2 is a recognized negative regulatory loop of RAS signaling that is positioned downstream of MAPK.[26] Inactivation of RSK2 resulted in the release of this negative feedback and constitutive activation of the pathway.[12] Experimental results further imply that prolonged RAS activation may be a cause of sorafenib resistance in HCC.[12]

miRNA dysregulation has a well-established role in the PI3K-AKT-mTOR pathway. miR-660-5p, miR-1914, and miR-125a-5p, moreover, noncoding miRNA, activated PI3K/AKT signaling to promote the proliferation and invasion of HCC cells.[14] The major tumor suppressor and oncogenic miRNAs and their related genes and pathways are summarized in Table 1.

Table 1. Tumor-suppressor and oncogenic miRNAs and their regulated gene expressions

		References
Tumor supressor miRNAs	**Regulated gene expressions**	
miR-206	cMET, CCND1 and CDK6	[27]
miR-214	EZH2, β-catenin	[28, 29]
miR-223	STMN1	[30]
miR-302b	AKT2	[31]
miR-339	ZNF689	[32]
miR-15a, miR16 and miR-107	WNT3A	[33]
miR-375	YAP and AEG-1	[34]
miR-424-5p	TRIM 29	[35]
miR-501-3p	LIN7A	[36]
miR-874	DOR, EGFR and ERK	[37]
miR-140	MMP9	[38]
Oncogenic miRNAs	**Regulated gene expressions and/or pathways**	
miR-1914	PI3K/Akt	[39]
miR-660-5p	PI3K/Akt	[40]
miR125a-5p	PI3K/Akt	[41]
miR-3131	DTHD1 and XAF1	[42]
miR-122	Induced HCV replication	[43]
miR-17-5p	PTEN, GalNT7, Vimentin	[44]
miR-18a	ESR1	[45]
miR-32	PTEN	[46]
miR-92b	SMAD7	[47]
miR-93	PTEN, CDKN1A, c-Met/p13k/AKT pathway	[48]
miR-101	FOS oncogene	[49]
miR-106b	EMT	[50]
miR-130b	PPAR-γ, TP53INP1	[51]
miR-155	c-Myc, C/EBPβ, APC, β-catenin, Cyclin D1, TP53INP1	[52]
miR-197	Wnt/β-catenin, Axin-2, NKD1 and DKK2	[53]
miR-219-5p	Cadherin 1	[54]
miR-221 and miR-222	PHF2, AKT pathway, PTEN, CDK inhibitor p27 and DDIT4	[55]

cMET: Cellular mesenchymal epithelial transition; CCND1: Cyclin D1; EZH2: Enhancer of zeste homolog 2; STMN1: Stathmin 1; AKT 2: AKT Serine/Threonine Kinase 2; ZNF: Zinc Finger Protein 689; WNT3A: Wnt family member 3A; YAP: Yes-associated protein; TRIM 29: Tripartite motif containing 29; LIN7A: Lin-7 homolog A; DOR: Delta opioid receptor; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase; MMP9: matrix Metallopeptidase 9; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; DTHD1: Death domain containing 1; XAF1: XIAP associated factor 1, HCV: Hepatitis C virus; PTEN: Phosphatase and tensin homolog; GalNT7: Polypeptide N-Acetylgalactosaminyltransferase 7; ESR1: Estrogen Receptor Alpha Gene; SMAD7: SMAD Family Member 7; CDKN1A: Crosstalk of Cyclin-dependent kinase inhibitor 1A; p13k: phosphatidylinositol 3-kinase; FOS: Fos proto-oncogene, AP-1 transcription factor subunit; Epithelial-mesenchymal transition; PPAR-y: Peroxisome proliferator-activated receptor gamma; TP53INP1: Tumor protein p53 inducible nuclear protein 1.

Environmental factors, in addition to mutations, have an impact on the mTOR pathway activity. Under hypoxic conditions, HIF-2α was upregulated in steatotic HCC, resulting in activated PI3K and increased levels of phosphorylated AKT, GSK3B, and mTOR.[26] In 5–15% of instances, activating mutations of NRF2 (coded by NFE2L2) or inactivating KEAP1 changes the oxidative stress pathway by preventing the proteasome from degrading NRF2, which is physiologically driven by KEAP1/CUL3 complex ubiquitinoylation.[56] Activating the NRF2 pathway has been proven to shield mice from long-term oxidative stress and the development of tumors. NRF2 activation drives tumor growth by recurrent mutations in HCC.[57] *In vitro* studies have shown that activating NRF2 protects tumor cells against ROS exposure, which may otherwise result in death.[57]

CNVs and related mutations are prevalent genetic occurrences in HCC. Broad genomic deletions and insertions affecting 1p, 4p-q, 6q, 8p, 13p-q, 16p, 17p, 21p, 22q, and at 1q, 5p, 6p, 8q, 17q, 20q, and Xq have been found.[12,14,56] Genes, such as AXIN1, CDKN2A/CDKN2B, CFH, IRF2, MAP2K3, PTEN, PTPN3, RB1, and RPS6KA3, were damaged by recurrent homozygous deletions, whereas high-level focal amplifications affected the loci for VEGFA (1%) and FGF3/4/19/CCND1 (4%) on 6p21 and 11q13, respectively.[56,58] Fluorescence in situ hybridization was used in further studies to confirm the elevated levels of circulating tumor DNA in these loci.[58] JAK3, MET, and MYC have all been linked to larger DNA increases (3%, 1%, and 1%, respectively).[58] VEGFA amplifications provide a non-cell autonomous mechanism for sorafenib sensitiveness.[59] The major signaling pathways in HCC pathophysiology are represented in Figure 1.[60] Genetic interactions among the potential genes in HCC pathophysiology mentioned above are shown via GENEMANIA in Figure 2.

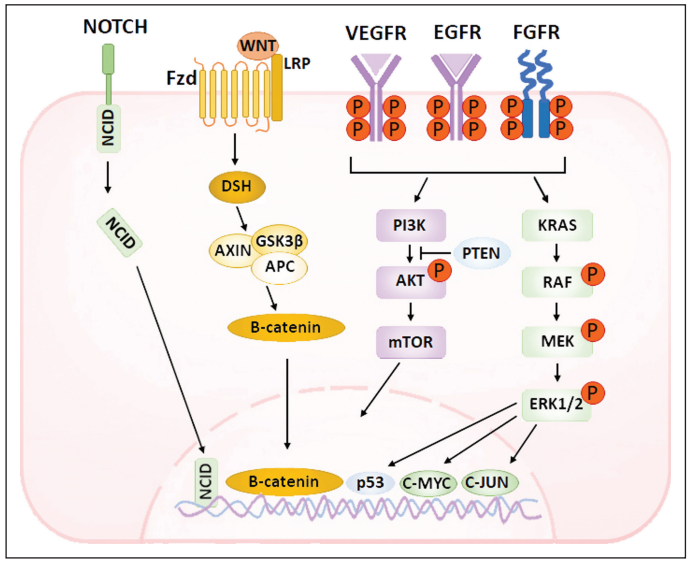

Figure 1. The major signaling pathways in HCC pathophysiology.

The Epigenetic Mechanisms of HCC

Epigenetic studies have shown altered patterns of methylation, as well as impairments in the enzymes involved in the DNA methylation process in HCC. Additionally, various histone modifications, such as de/methylation and de/acetylation, regulate gene expression reversibly. HCC cells are additionally affected by the impaired functionality of non-coding RNAs, including micro RNAs and long non-coding RNAs. Furthermore, there is increasing evidence indicating the involvement of liver cancer stem cells in the formation of HCC.

DNA Methylation Patterns in HCC

Methylation predominantly occurs in regions characterized by low CpG density or in repetitive DNA sequences.[61] In most cases, gene promoter methylation leads to transcriptional repression, but methylation occurring in the gene body tends to enhance gene expression in HCC.[62]

An abnormal DNA methylation pattern has been identified as a significant epigenetic mechanism that contributes to the development of HCC. Promoter sequences of genes involved in cell cycle regulation, apoptosis, DNA repair, metabolism of carcinogens, and angiogenesis are typically susceptible to aberrant hypermethylation in various regions. In HCC, there are three distinct forms of DNA methylation alterations: hypermethylation of CpGs in the promoter regions of tumor suppressor genes, aberrant expression of DNA methyltransferases (DNMTs), and global hypomethylation of genes and repetitive sequences, which leads to genomic instability and oncogene activation.[63]

DNA methylation alterations can be observed in the form of hypermethylation in tumor suppressor genes, such as CDKN2A, RASSF1, APC, and SMAD6.[62,64] Tumor suppressor gene APC is inactivated due to hypermethylation in most human HCC cases. This leads to the abnormal accumulation of β-catenin in cell nuclei, activating its oncogenic properties. Another gene affected by aberrant methylation in HCC is the cell cycle regulator p16INK4A. Multiple studies have shown frequent methylation of the p16INK4A gene in most human HCC cases. The absence of p16INK4A promotes abnormal cell proliferation. Since it can be detected not only in tissues but also in the serum of patients, it may serve as a potentially valuable marker for early HCC diagnosis.[65]

Studies have shown the role of abnormal expression of DNMTs in the development of HCC. A methyl group is transferred to the 5-carbon of cytosine by DNMTs. DNMT1, DNMT3A, and DNMT3B play a role in the development and progression of cancer. DNMT1 is primarily active during cell division for maintenance, while DNMT3A and DNMT3B are responsible for de novo methylation of DNA during cellular differentiation.[66]

In HCC, hypomethylation activates proto-oncogenes, such as c-Jun and c-myc,[67] potentially enhancing carcinogenesis by affecting mitotic recombination and increasing genomic instability.[68]

Histone Modifications in HCC

Histone acetyltransferases (HATs) add acetyl groups to histone side chains, loosening the interaction between histones and chromatin, while histone deacetylases (HDACs) remove these acetyl groups, stabilizing chromatin structure and limiting access to transcription factors. These modifications can influence cancer development by altering the expression of oncogenes, tumor suppressor genes, and chromatin structure. In HCC, HDAC1 is associated with moderately and poorly differentiated tumors, and HDAC2 is a negative prognostic factor for survival.[69] HDAC2 is involved in the epigenetic regulation of the cell cycle, apoptosis, and differentiation in HCC.[70] HDAC3 is also implicated in HCC development and regulates the cell cycle and proliferation. Silencing HDAC2 and HDAC3 has been shown to inhibit HCC growth, and their functions are linked to various cellular processes and pathways, including the regulation of histone acetylation, STAT3 signaling, and double-strand break repair.[71] Additionally, HDAC1 and HDAC3 jointly regulate cell migration, EMT, and tumor metastasis by altering the expression of certain genes involved in these processes.[72]

The methylation of lysines on histones is a crucial aspect of epigenetic histone modifications and controlled by various enzymes, including methyltransferases and demethylases. In HCC, there is often abnormal regulation of these enzymes, leading to alterations in gene expression. A recent study identified 11 methyltransferases and demethylases, including EZH2, EHMT2, SETDB1, and SETD2, which were associated with the clinical characteristics of HCC tissues compared to normal liver tissues. This finding underscores the significant role of histone methylation regulation in HCC pathogenesis and highlights its potential as a therapeutic target.[73]

The major sites of histone methylation include lysine 4, 9, and 27 of histone H3. Histone 3 lysine 4 methylation (H3K4me) is generally associated with gene activation, while histone 3 lysine 9 methylation (H3K9me) and histone 3 lysine 27 methylation (H3K27me) lead to gene repression. SET7 is a specific methyltransferase responsible for methylation of H3K4 and is implicated in HCC.[74,75]

Non-coding RNAs in HCC

Non-coding RNAs, particularly miRNAs, play a crucial role in HCC. miRNAs such as let-7, miR-34a, miR-221, miR-222, and miR-122 are involved in HCC pathogenesis. miR-221/222 is upregulated in HCC tumor samples and enhances cell growth by targeting p27 and DDIT4. Other miRNAs like miR-369, miR-3174, miR-383, and miR-361-5p are linked to HCC cell proliferation, apoptosis, and chemoresistance. miR-186 affects liver cancer stem cells and chemoresistance. Therapeutic strategies targeting specific miRNAs using synthetic inhibitors have shown promise in inhibiting tumor growth.[55,76] The major tumor suppressor and oncogenic miRNAs and their related genes and pathways are summarized in Table 1.

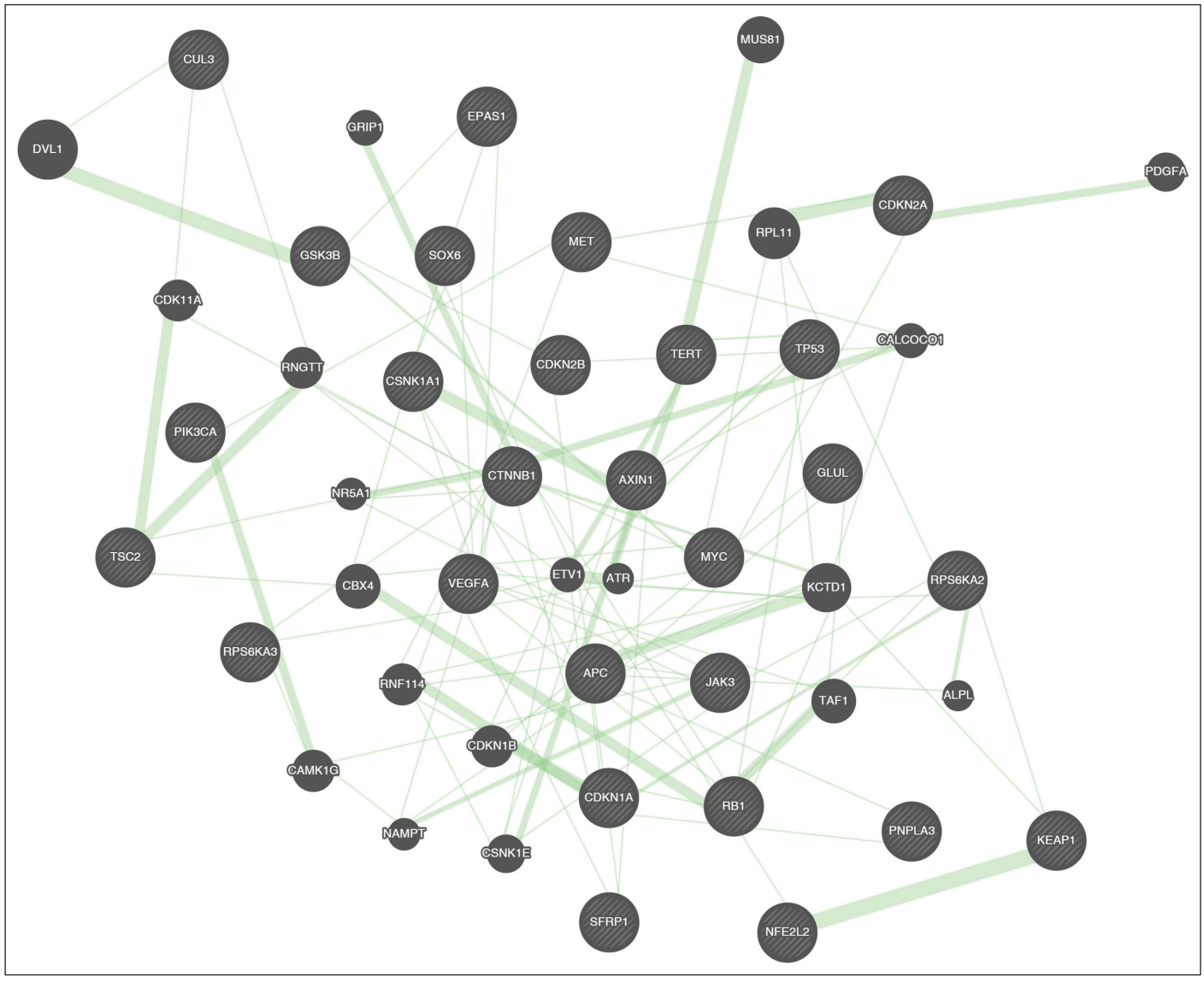

Figure 2. Network of the genetic interactions of the potential genes in HCC pathogenesis.

Long non-coding RNAs (lncRNAs) also play a significant role in HCC development. LncRNAs like HOTAIR, HOTTIP, MALAT1, HULC, NEAT1, ANRIL, SNHG1, and H19 are implicated in HCC proliferation, metastasis, and chemosensitivity.[77–79] Targeting dysregulated lncRNAs presents potential therapeutic avenues for HCC. However, challenges in RNA interference-based therapies must be overcome for effective treatment.[80]

Biomarkers of HCC

Circulating biomarkers serve three commonly cited roles: clinical diagnosis, monitoring disease progression, and assessing prognosis. For example, circulating nucleic acid biomarkers have been shown to be viable and applicable for the early detection of HCC. The ideal biomarker possesses certain universal characteristics for routine clinical analysis, including being sensitive, specific, easy to use without requiring extensive operator experience, cost-effective, highly reproducible, providing rapid results, correlating with tumor stages, and utilizing readily available sample sources.[81]

AFP is one of the most used biological markers in the diagnosis and monitoring of HCC. AFP is primarily produced during fetal development and its synthesis typically ends after birth. However, in the context of liver cancer, particularly HCC, AFP synthesis becomes highly expressed. AFP serves as a carrier protein for various metabolites, such as bilirubin, steroids, fatty acids, and certain drugs. It circulates in the bloodstream and can be measured through a blood test. To detect HCC, a commonly used cutoff level for AFP is set at more than 400 U/L. However, it's important to note that while AFP is a marker for HCC, its sensitivity and specificity can vary.

AFP can be further categorized into three isoforms based on its ability to bind to the lectin Lens culinaris agglutinin (LCA). These isoforms include:

AFP-L1 (nonbinding fraction): AFP-L1 is the isoform that does not bind to LCA.

AFP-L2 (weak binding fraction): AFP-L2 has a weaker affinity for lectin binding compared to AFP-L3.

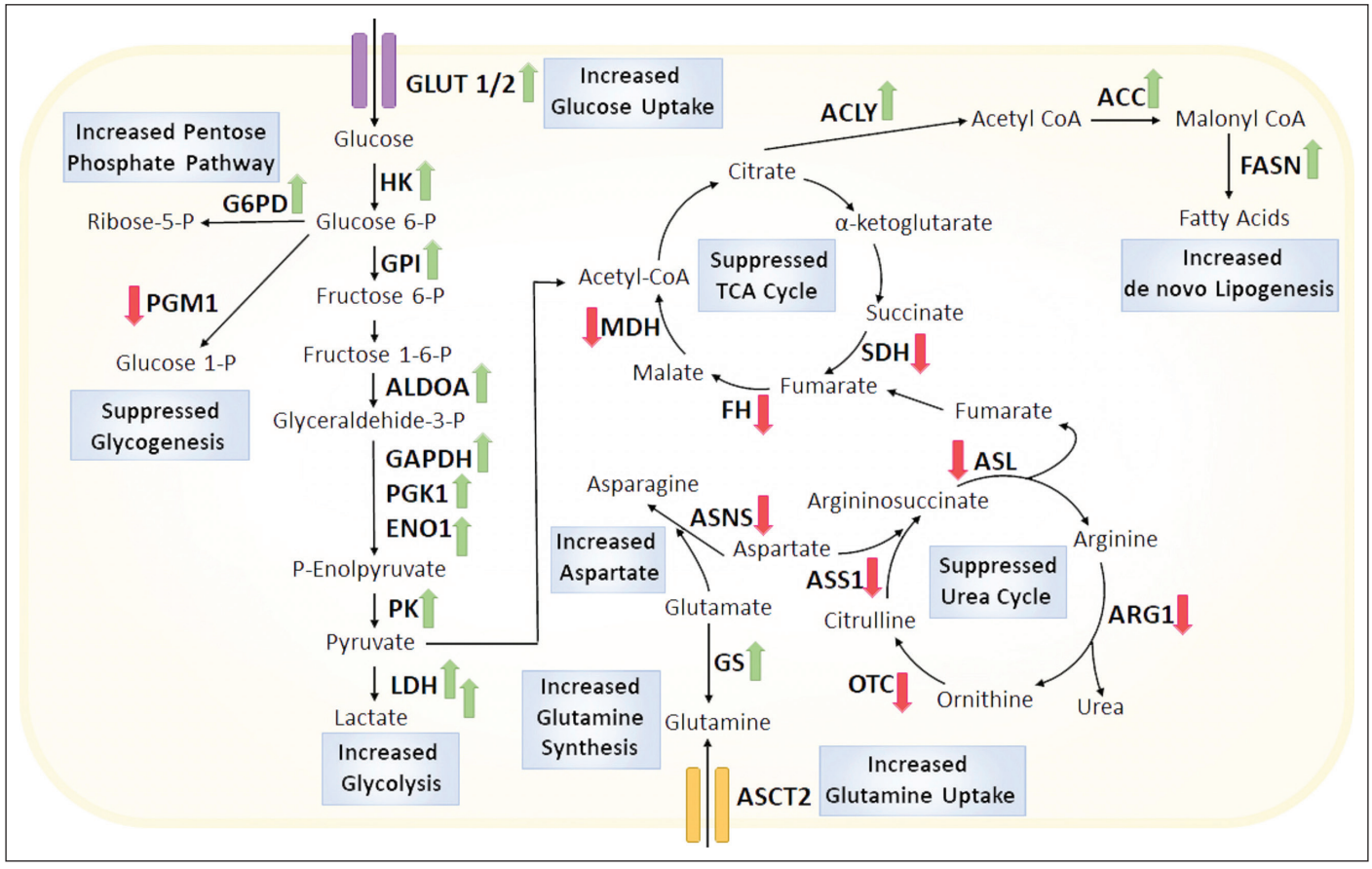

Figure 3. The major alterations in metabolic pathways in HCC.

AFP-L3 (binding fraction): AFP-L3 is the isoform that specifically binds to LCA. Importantly, AFP-L3 is primarily elevated in individuals with HCC. This characteristic makes AFP-L3 a valuable diagnostic biomarker for HCC.

In clinical practice, the measurement of high-sensitivity AFP-L3 (hs-AFP-L3) can be useful for the early detection of HCC, as it is more specific to HCC compared to total AFP. While the sensitivity of total AFP for HCC detection can range from 20% to 60%, the specificity can range from 80% to 100%. The use of AFP-L3, in combination with other diagnostic tools, can aid in the early identification of HCC.[82]

Glypican-3 (GPC3) is a proteoglycan that is present on the cell surface and is expressed in most cases of HCC, but not in normal or cirrhotic liver tissues. Immunostaining for GPC3 is commonly used in diagnostic pathology to confirm the diagnosis of HCC. GPC3 is also being explored as a potential target for the treatment of HCC, with ongoing clinical trials.[83]

Several protein antigens, including MMPs, such as MMP-1 and MMP-15, and glutamine synthetase (GS), have been associated with HCC. Selective MMP inhibitors show potential in halting the spread and growth of HCC.[84] Alpha-L-fucosidase (AFU) and paraoxonase 1 (PON1) have also been proposed as novel biomarkers for HCC diagnosis, with PON1 showing promise as a consistent marker.[85,86]

Furthermore, growth factors and their receptors, such as transforming growth factor beta (TGFβ), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF), play essential roles in hepatocarcinogenesis. These factors are frequently dysregulated in HCC, contributing to tumor growth, angiogenesis, and metastasis.[87,89]

Circulating Tumor Cells (CTCs)

In addition to the previously mentioned markers, circulating nucleic acid markers, including cell-free DNA (cfDNA), are gaining prominence as biomarkers for HCC through liquid biopsy. cfDNAs are DNA molecules that are released from cells into bodily fluids such as blood, urine, and cerebrospinal fluid through processes like apoptosis, necrosis, and active secretion.[90] Additionally, subpopulations of cfDNA molecules, such as cell-free mitochondrial DNA (mtDNA), cell-free viral DNA, cfRNA, and extracellular vesicles (EVs), also serve as informative biomarkers in oncology. These markers offer promising avenues for early detection, monitoring, and personalized treatment of HCC and other cancers.[91]

Alterations of Metabolic Pathways in HCC

Metabolic alterations are the common characteristics of cancer cells giving a selective advantage for tumor growth, proliferation, and survival by supplying the critical demands of rapidly growing cancer cells, such as enhanced energy production and accelerated biosynthetic pathways.[92] The liver is the main site of numerous metabolic events which govern energy metabolism by the regulation of various metabolites, including sugars, lipids, and amino acids. Therefore, it has been indicated that HCC is accompanied by a highly dysregulated metabolism compared to normal hepatocytes.[93,94] Aside from causing metabolic reprogramming, abnormalities in metabolic pathways also remodel the

tumor microenvironment through different metabolic signaling pathways. Thus, this synergistically promotes the occurrence and progression of tumors.[95] However, the increased reliance of the tumor on some of these pathways might result in metabolic vulnerabilities in HCC, which can be targeted by inhibitors of these pathways.[94] Therefore, it is crucial to understand the metabolic alterations in HCC cells and the underlying mechanisms behind these alterations on gene and protein levels, as well as their crosstalk with the other signaling pathways, for improving future potential therapeutic interventions.

Altered Carbohydrate Metabolism in HCC

Under physiological conditions, glucose is converted into pyruvate by glycolysis and then completely oxidized in the mitochondria by the tricarboxylic acid (TCA) cycle and phosphorylation. Otherwise, it can be channeled in the fatty acid synthesis pathway through de novo lipogenesis. However, studies have shown that HCC predominantly displays the Warburg effect with quick energy generation from glycolysis, through the conversion of glucose to lactate, instead of allowing pyruvate to enter the TCA cycle even in the presence of oxygen and fully functioning mitochondria.[96] While glycolysis enzymes are primarily upregulated in HCC, glycogen metabolism enzyme phosphoglucomutase 1 (PGM1) and gluconeogenesis enzymes phosphoenolpyruvate carboxykinases 1 and 2 (PEPCK), and fructose 1,6-bisphosphatase 1 (FBP1) are downregulated. This leads glucose to undergo glycolysis instead of being stored as glycogen and causes a loss of the gluconeogenic activity of normal liver cells.[94] Further, HCC tumors enhance glucose uptake by upregulating plasma membrane glucose transporter 1 (GLUT1) and GLUT2.[97,98] Accordingly, the TCA cycle is downregulated and due to the increased flux of glycolytic metabolites through the pentose phosphate pathway (PPP), nucleotide metabolism is promoted.[99,100] The major dysregulated pathways of carbohydrate metabolism and their compounds in HCC are presented in Figure 3.[101] Overall, this metabolic reprogramming promotes the growth, survival, proliferation, and long-term maintenance of HCC cells. Therefore, targeting glucose metabolism may serve as a basis for the development of potential drugs in HCC treatment.

Altered Lipid Metabolism in HCC

The liver is the central organ for fatty acid metabolism, which synthesizes, stores, processes, and breaks down lipids. Therefore, in HCC pathogenesis, several pathways of lipid metabolism are dysregulated, such as fatty acid synthesis, β-oxidation, and cellular lipidic composition, to support tumorigenesis. Studies have shown that the genes involved in fatty acid and cholesterol biosynthesis are upregulated, including mainly acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), ATP citrate lyase (ACLY), SCD1 (stearoyl-Coenzyme A desaturase 1), and cholesterol biosynthesis gene 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), as well as sterol regulatory element-binding protein 1 (SREBP1), an adipogenesis transcriptional regulator.[102] Not only are the fatty acid biosynthesis enzymes upregulated, but also glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME), which produce NADPH supplying the reducing equivalents for fatty acid biosynthesis enzymes.[41] In parallel to the upregulation of fatty acid biosynthesis, β-oxidation is suppressed by the downregulation of carnitine palmitoyl transferase 2 (CPT2) enzyme expression.[41,103] Catalyzing the first reaction of ketogenesis, the 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) enzyme has also been shown to be downregulated in HCC tumor tissues. Lower expression levels of HMGCS2 were correlated with tumor size as well as higher pathological grades, which suggests HMGCS2 may function as a tumor suppressor.[93,104] However, many metabolites are also associated with various signaling pathways in addition to their roles in energy metabolism. For instance, unsaturated fatty acids have been addressed to downregulate PTEN in hepatocytes through an mTOR/NF-κB-dependent mechanism.[105] Interestingly, an increasing number of studies have uncovered that apolipoproteins play a significant role not only in lipid metabolism but also in influencing tumor behavior and regulation.[106] Whole-exome sequencing analyses have shown APOB gene mutations in 10% of HCC tumors.[107] GWAS link APOB mutations to liver tumor growth by activating cancer-promoting genes and silencing tumor-suppressing genes, indicating its key regulatory function in cancer.[108] ApoA1 is found to suppress HCC by promoting cell cycle arrest and tumor cell apoptosis via inactivating the MAPK pathway.[109] ApoM can also suppress tumors by altering glycolysis in liver cancer cells via the SREBP1 pathway.[110] APOE is reported to be positively correlated with the HCC tumor grade.[111] Therefore, crosstalk should be considered in terms of discovering synergy among pathways and understanding the holistic effects of metabolic components. The major dysregulated pathways of lipid metabolism and their compounds in HCC are presented in Figure 3.[101]

Altered Amino Acid Metabolism in HCC

Amino acid metabolism has been shown to be altered in HCC. Both the amino acids and the related enzymes participate in providing energy and precursors to support tumor initiation and progression. One of the major alterations takes place in aspartate metabolism since the expression levels of asparagine synthetase (ASNS), the enzyme producing asparagine by using aspartate, are decreased in correlation with the malignancy of HCC tumors.[112] In HCC, other significant upregulation has been detected in glutamine metabolism with the overexpressed glutamine synthetase (GS).[94] Despite the increased expression of GS, the serum glutamine levels of HCC patients have been found to be lower than healthy individuals. This outcome is considered the result of increased glutamine uptake by tumors due to the upregulated glutamine transporter (ASCT2).[113,114] Proline metabolism was also markedly changed in HCC tumor tissue, characterized by accelerated consumption of proline and the accumulation of hydroxyproline.[115] However, these amino acids do not only contribute to biosynthesis but also interact with signaling pathways. For example, glutamine shows its tumor proliferative effects by activating mTORC1 signaling, and accumulated hydroxyproline promotes HCC tumor progression and sorafenib resistance by modulating HIF1α stability.[115] Additionally, the urea cycle, the process that excretes the byproducts generated by protein degradation, is significantly downregulated in HCC.[116] The major dysregulated steps of the urea cycle in HCC are presented in Figure 3.[101] Overall, these findings imply that targeting the affected metabolic pathways may potentially play an essential role in HCC therapy. Nevertheless, in HCC pathogenesis, it should be considered that the metabolic pathways crosstalk with each other. Therefore, the synergy of interconnected pathways should be evaluated instead of isolated mechanisms.

Conclusion

The importance of the liver as a vital complex regulatory system for homeostasis is based on crosstalk between biochemistry and genetics,

through the identification of several mutations of genes that impair biochemical pathways and enzyme/protein expressions at different levels with several clinical manifestations. The basic scientific understanding of the biochemical mechanisms linked to genetic variants controlling hepatic tumorigenesis, autoinflammation, and autoimmunity has progressed considerably during the past few years, thanks to advances in NGS and GWAS. The challenges for the future will be to understand how the crosstalk between biochemistry and genetics affects hepatic function and the pathogenesis of liver diseases linked to hepatic tumorigenesis, autoinflammation, and autoimmunity. The aim of this review is to update how the crosstalk between genetics and biochemistry affects the pathogenesis of the most common liver diseases. Genetically affected biochemical pathways, both major survival paths of signaling, such as RAF/MEK/ERK, Wnt/β-catenin, PI3K/AKT/mTOR, JAK/STAT, EGFR, and MET, and main metabolic paths of biomolecules, are evaluated in details. Some of these important pathways can be novel diagnostic/prognostic biomarkers, and/or novel therapeutic markers for the treatment of these liver diseases. Besides, by unveiling novel genetic variants in hepatic pathogenesis, this review points to the mechanisms that link these genetic variants to impaired biochemical paths, highlighting the importance of several genetic variants and the complexity of biochemical pathways' crosstalk in the regulation of hepatocellular homeostasis.

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