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HEPATIC CANCER (N PARIKH, SECTION EDITOR)

Genomic Landscape of HCC

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Abstract

Introduction Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality in the world, and it has limited treatment options. Understanding the molecular drivers of HCC is important to develop novel biomarkers and therapeutics.

Purpose of Review HCC arises in a complex background of chronic hepatitis, fibrosis, and liver regeneration which lead to genomic changes. Here, we summarize studies that have expanded our understanding of the molecular landscape of HCC.

Recent Findings Recent technological advances in next-generation sequencing (NGS) have elucidated specific genetic and molecular programs involved in hepatocarcinogenesis. We summarize the major somatic mutations and epigenetic changes have been identified in NGS-based studies. We also describe promising molecular therapies and immunotherapies which target specific genetic and epigenetic molecular events.

Summary The genomic landscape of HCC is incredibly complex and heterogeneous. Promising new developments are helping us decipher the molecular drivers of HCC and leading to new therapies.

Keywords Liver cancer · HCC · Carcinogenesis · Mutation · Genomics · Therapeutics

Abbreviations

2D LC-MS/MS	Multidimensional liquid chromatography-tandem mass spectrometry
AFP	Alpha-fetoprotein
ALD	Alcoholic liver disease
ALL	Acute lymphoblastic leukemia
ATM	Ataxia telangiectasia mutated
CDH1	Cadherin-1
CNV	Copy number variations
DFS	Disease-free survival
DNMTs	DNA methyltransferases
FDA	Food and Drug Administration
GOF	Gain of function
HATs	Histone acetyltransferases
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
hCSC	Hepatic cancer stem cell
HCV	Hepatitis C virus
HDACs	Histone deacetylases
HDAC3	Histone deacetylase 3
JAKs	Janus kinases
lncRNAs	Long non-coding RNAs
LT	Liver transplantation
MAT1A	Methionine adenosyltransferase 1
MMP9	Matrix metalloproteinase-9
miRNAs	MicroRNAs
NAFLD	Nonalcoholic fatty liver disease
NGS	Next-generation sequencing
NF-KB	Nuclear factor-? beta
OS	Overall survival
PLEC1	Plectin 1
PRC1	Protein regulator of cytokinesis 1
SCNAs	Somatic copy number alterations
scRNA-Seq	Single-cell RNA sequencing
TACE	Transarterial chemoembolization
TCGA	The Cancer Genome Atlas
TERC	Telomerase RNA component
TERT	Telomerase reverse transcriptase
TF	Transcription factor
TOP2A	Topoisomerase 2 α
JAKs	Transmembrane receptor Janus kinases

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Introduction

The global incidence of hepatocellular carcinoma (HCC), the most common primary liver malignancy and the 6th most common cancer worldwide, is expected to significantly increase over the next 10 years [1, 2]. Unfortunately, HCC survival still remains dismal, with 5-year survival rates of 32.6%, 10.8%, and 2.4% for localized, regional, and distant stages of disease, respectively [3]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common risk factors for HCC, and the incidence of HCC has historically mirrored the incidence of these infectious diseases [4]. With the advent of the HBV vaccine and HCV antiviral therapy, there is hope that the burden of hepatitis-related HCCs will decrease. However, viral hepatitis is still expected to drive increased incidences of HCC over the next 10 years [2, 5]. Furthermore, alcoholic liver disease (ALD), obesity, and nonalcoholic fatty liver disease (NAFLD) remain important risk factors for HCC, and these etiologies are actually increasing in incidence [6, 7].

Even though risk factors for HCC vary from region to region, the mechanisms of hepatocellular carcinogenesis mostly converge on the processes of chronic liver inflammation and regeneration. Chronic liver injury secondary to either virus-induced inflammation, alcohol-induced hepatocellular damage, or lipotoxicity-induced oxidative stress lead to a vicious cycle of regeneration and fibrosis that increases the risk of genomic instability and hepatocarcinogenesis [8, 9]. These pathogenic mechanisms underscore why 80–90% of HCC arise in a cirrhotic liver [10]. However, HCC can also arise in the non-cirrhotic liver, especially in patients with HBV or NAFLD [11, 12]. Thus, the genomic landscape in which hepatocarcinogenesis occurs is incredibly complicated.

Currently, the only definitive therapeutic cure for HCC is liver transplantation (LT), and even LT is associated with recurrence rates of 10–15% [13]. For unresectable tumors, few therapies exist. The oral multi-kinase inhibitor sorafenib has been the therapeutic workhorse for unresectable HCCs ever since it was approved by the Food and Drug Administration (FDA) in 2007. However, its therapeutic efficacy has been greatly limited by rapid drug resistance and toxicities [14, 15]. Despite the recent approval of new first line therapies like atezolizumab/bevacizumab or lenvatinib and second-line therapies like regorafenib, nivolumab, and cabozantinib, there still remains a pressing need for effective therapeutics that can significantly improve long-term survival [16–18].

Genomic Landscape of HCC

The dearth of therapeutic options for HCC continues to propel research into the mechanisms of hepatocarcinogenesis. Over the last 5 years, significant progress has been made in the identification of somatic mutations, copy number variations (CNVs), and epigenetic modifications that drive hepatocarcinogenesis and contribute to disease outcomes (Table 1). Improvements in genome-wide screening and high-throughput genomics have led to the identification of new gene signatures and proteomic targets that can help to diagnose and prognosticate HCC. Recent developments in single-cell RNA sequencing (scRNA-Seq) are opening a window into the pathophysiology of tumor heterogeneity. This review summarizes the genomic landscape of HCC (Fig. 1) and identifies studies that have recently expanded our understanding of hepatocarcinogenesis in a meaningful way.

Somatic Mutations

The normal aging liver is thought to acquire 30–40 somatic mutations per year, either induced by genotoxic stress or random mutations arising from DNA replication [78, 79]. Hepatic stem cells and differentiated hepatocytes have both been shown to acquire these mutations, with a recent study revealing that mature hepatocytes in the normal liver have twice the rate of somatic mutations as hepatic stem cells [80]. Still, compared to other tissues heavily dependent on stem cell regeneration, the overall mutational burden and malignant potential of the normal adult liver remains low [78, 79]. In the setting of chronic liver disease and inflammation, however, hepatocytes are susceptible to additional proliferation-induced mutagenesis by way of mitochondrial damage and oxidative/endoplasmic reticulum stress [81]. Another mechanism of mutagenesis is via genomic viral integrations as seen in HBV-HCCs, which typically have the highest rates of somatic mutation [82]. HCV, on the other hand, typically promotes HCC carcinogenesis through double strand breaks that result in missense mutations. Lastly, somatic mutations in NAFLD, ALD, and toxin-driven HCCs are typically caused by direct DNA damage through chronic inflammation and reactive oxygen species.

Clinically significant driver mutations in HCC have been shown to involve these major pathways: tumor suppressor genes (*TP53, ARID 1/2, RB1, TSC1/2*), telomerases (*TERT/TERC*), the Wnt/β-catenin pathway (*CTNNB1, AXINI, AXIN2*), PI3K/Akt/mTOR pathways, MYC pathway, JAK/STAT pathways (*JAK1, IL6R, IL6ST*), oxidative stress pathways (*KEAP1, NFE2L2*), RAS/RAF/MAP kinases (*RPS6KA3*), and the MET pathway. We will now discuss the mutations in these major pathways in further detail.

Tumor Suppressor Genes

As the "guardian of the genome," the *TP53* tumor suppressor gene is responsible for the regulation of cellular processes like cell death and angiogenesis. It is the most frequently altered

Table 1 Overview of major genomic landscape and clinicopathologic correlations in HCC

Type of genomic change	Class	Main gene target	References	Clinicopathologic associations	Therapeutic relevance
Somatic mutations	Tumor suppressors	TP53 (p63, p73, p14ARF, mdm2, mdm4, ATM)	Teramoto et al., 1994 [84] Chan et al., 2004 Villanueva et al., 2011 Chittmittrapap et al. 2013 [86] Zhan et al., 2013 Zhan et al., 2014 Torrecilla et al., 2017 [27] Long et al., 2019 [23] Li et al., 2020 [93]	Clinical: HBV-HCC (19) Histopathology: increased stem cell markers (20), high Edmonson grade (19, 21), decreased in areas of cholestasis and tumor infiltrating lymphocytes (19), increased in areas of necrosis (19) Survival: high recurrence rates, poor OS (19, 20, 22, 23) and DFS (19, 22), downregulated immune responses (24)	Resistance: doxorubicin (25, 26)/paclitaxel (25) (in vitro) Therapeutics: p53 MVA vaccine + pembrolizumab (phase I, 40), recombinant adenovirus p53 (phase II, 41, 43, 44)
		ARID1A ARID1B	Schulze et al., 2015 Nahon et al., 2017 Hu et al., 2018 [95] Qu et al., 2019 [24] Moore et al., 2019 [91] Li et al., 2020 [93] Peng et al., 2020 [94]	Clinical: ALD-HCC (27), HBV-HCC (27–29), tobacco use (27) Histopathology: hepatic steatosis (30, 31), larger more aggressive tumors (32, 33), increased angiogenesis (34), poor OS (32, 33), mismatch repair (33)	–
	Telomerases	TERT promoter	Nault et al., 2013 [30] Nault et al., 2016 Pezzuto et al., 2016 [34] Torrecilla et al., 2017 [27] Yu et al. 2017 [117] Pezutto et al. 2017 Kawai-Kitahata et al., 2017 Li et al., 2018 [98] Cheng et al., 2019 [97] Li et al., 2020 [93]	Clinical: older age, African or European ancestry, HCV-HCC, HBV-HCC (35–37) Histopathology: low/high grade dysplastic nodules (38, 39) Survival: shorter DFS (40–42), late intrahepatic recurrence (40)	Therapeutics: INO-1400 or INO-1401 ± INO-9012 (phase I, 66)
	Growth factor pathways	*CTNNB1 (Wnt/β-catenin) (APC, AXIN1, AXIN2, PRC1, AKIP1, TXNDC12, DICER1)*	Zulehner et al., 2010 [33] Schulze et al., 2015 Torrecilla et al., 2017 [27] Ataide et al., 2017 [37] Li et al., 2020 [93]	Clinical: ALD-HCC (28) Histopathology: micro- and macrovascular invasion, increased pathologic grade, increased tumor size, and multifocal disease (43, 44) Survival: HCC recurrence (43, 44)	Therapeutics: DKK1 antibody (phase I/II, 79)
	Signal transduction	*RPS6KA3 (MAP/ERK)*	TCGA, 2017 Calderaro et al., 2017 [105] Doycheva et al., 2019	Histopathology: poor differentiation, macrovascular invasion, high proliferation, and chromosomal instability, multifocal disease (45–47)	–
		STAT3 (JAK/STAT)	Kan et al., 2013 [111]		

Table 1 (continued)

Type of genomic change	Class	Main gene target	References	Clinicopathologic associations	Therapeutic relevance
				Histopathology: promotes proliferation, angiogenesis, metastasis, Warburg effect, inhibition of apoptosis	Therapeutics: STAT inhibitors: OPB-111077 (phase I, 97), OPB-31121 (phase I, 98), Napabucasin (phase I, 99), and AZD9150 (phase 1b, 100)
	Other	*KEAP1*	Zheng et al., 2019 [107]	–	Resistance: acquired resistance to sorafenib, lenvatinib, and regorafenib (48)
		HNF1A	Reznik et al., 2004 [108] Rebouissou et al., 2007 [44] Zeng et al., 2011 [109] Hetchman et al., 2019	Clinical: negative viral status, female sex, no cirrhosis (49–51) Histopathology: reduced expression associated with proliferation, migration, and invasion in HCC cells (52) Forced expression leads to differentiation into mature hepatocytes (52, 53)	–
CNVs	Gene amplification	*MYC*	Chan et al., 2004 Poon et al., 2006 Schlaeger et al., 2008 [115] Pedica et al., 2013 [118] Ao et al., 2016 [116] Yu et al., 2017 [117]	Clinical: viral hepatitis, ALD (54, 55) Histopathology: large undifferentiated tumors (56–59) Survival: poor DFS, metastasis, HCC recurrence (56–59)	
		E2F1/E2F3	Kent et al., 2017 [119]	Histopathology: advanced HCC (60)	–
		MMP9	Yu et al., 2020 [120]	Clinical: AFP level (61) Histopathology: tumor size, differentiation, invasion, stage (61)	–
		UBE2Q1	Zhang et al., 2018 [123]	Survival: poor OS and DFS (62)	–
		EXT1	Dong et al., 2018 [124]	Clinical: AFP level (63) Survival: poor DFS (63)	–
		WNK2	Zhou et al., 2019 [125]	Histopathology: increased tumor growth, macrophage infiltration (64) Survival: early tumor recurrence, metastasis (64)	–
		FGF19	Kaibori et al., 2016 [126]	Histopathophysiology: aggressive, undifferentiated tumors (in vivo) (Sawey et al., 2012), increased proliferation/invasion (in vitro) (Miura et al., 2012) Survival: poor overall and disease free survival (Miura et al., 2012)	Resistance: complete response after sorafenib treatment
	Gene deletions	*CDKN2A*	Kawai-Kitahata et al., 2016 [121] Cancer Genome Atlas Research Network et al., 2017 [104]	Histopathology: cell cycle arrest in G1/G2 Survival: poor prognosis, advanced aggressive cancer	–
		RB1	Kawai-Kitahata et al., 2016 [121]	Clinicals: HBV/HCV HCCs (65)	–

Table 1 (continued)

Type of genomic change	Class	Main gene target	References	Clinicopathologic associations	Therapeutic relevance
			Cancer Genome Atlas Research Network et al., 2017 [104] Sun et al., 2019 [122]		
Epigenetic modifications	DNA methylation	*APC*	Um et al., 2011 Shen et al., 2013	–	Treatment: 1st-line: azacitidine and decitabine, 2nd-line: guadecitabine and zebularine (phase I/II, 126–127)
		GSTP1	Shen et al., 2013	Clinicals: HBV	
		RASSF1a	Shen et al., 2013	Clinicals: aflatoxin B1	
		p16	Um et al., 2011	Clinicals: aflatoxin B1	
		COX2	Um et al., 2011	Histopathophysiology: dysplastic nodules (66)	
		CDH1	Wu et al., 2019 [56]	Survival: poor OS (67)	
	miRNAs	*mIR21*	Xu et al., 2013	Histopathology: HCC proliferation and migration (68–73)	–
		mIR221/222	Pineau et al., 2010		Treatment: miravirsen (phase II, 137–138)
		mIR224	Lin et al., 2016		–
		mIR-26	Yang et al., 2014	Histopathology: suppression of HCC proliferation and angiogenesis (68–73)	–
		mIR-122	Coulouarn et al., 2009		–
		mIR-199	Guo et al., 2015		–
		mIR 200a	Chen et al., 2017 [61]	Histopathology: inhibit cell growth, migration and invasion (74, 75)	–
	lncRNAs	*HULC*	Li et al., 2016 [63]	Histopathology: proliferation, EMT, angiogenesis (76)	–
		HOTAIR	Fujisaka et al., 2017 [64]	Histopathology: no impact on cell proliferation, migration (77), actively recruited macrophages	Resistance: chemoresistance (140,142)
	HDAC	*HDAC3*	Lu et al., 2018 [130] Ji et al., 2018	Histopathology: HCC proliferation (143–144) Survival: HCC recurrence, reduced DFS (143)	Treatment: Panobinostat, Belinostat, Resminostat, CUDC-101 (phase I/II, 148–150)
		HDAC1/2	Ler et al., 2015 Yang et al., 2019 [48]	Survival: predict mortality (145, 146)	Resistance: regulation doxorubicin sensitivity (145)

AFP alpha-fetoprotein, *ALD* alcoholic liver disease, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *HDAC* histone deacetylases, *OS* overall survival, *DFS* disease-free survival, *lncRNAs* long non-coding RNAs, *miRNAs* microRNAs

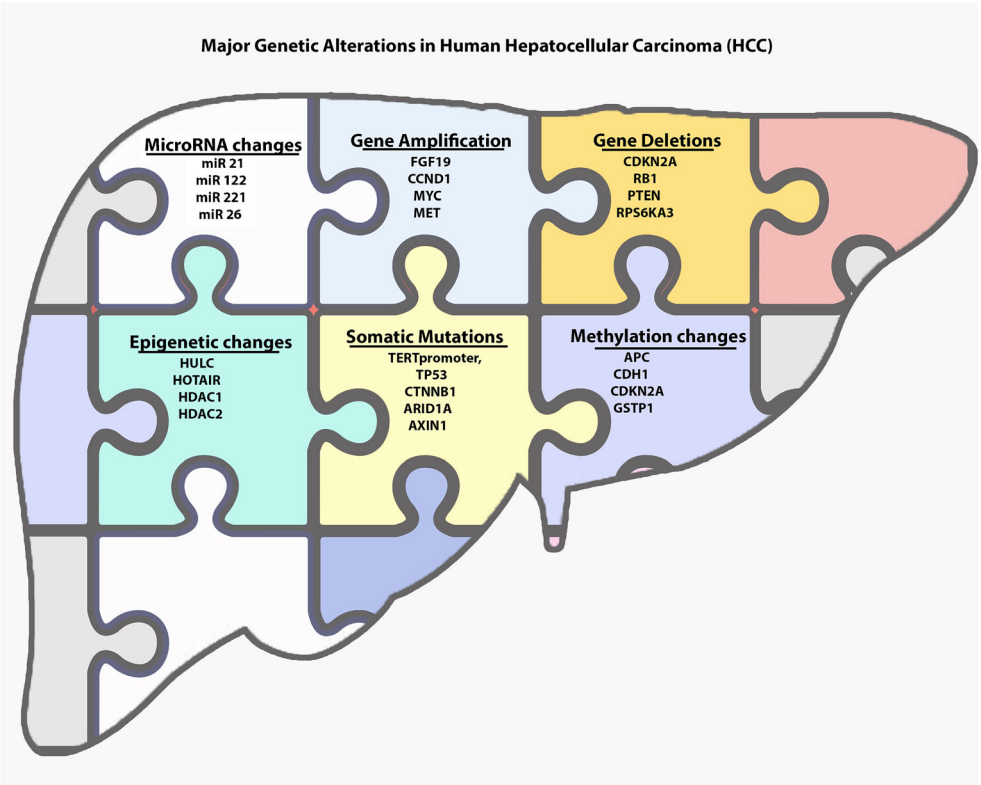

Fig. 1 Major genetic alterations in human hepatocellular carcinoma (HCC)

gene in human cancer, with the International Agency for Research on Cancer (IARC) reporting over 29,000 *TP53* mutations in human cancers [83]. *TP53* is altered or inactivated in 30–50% of all HCCs—80% of aflatoxin B1-HCCs, 45% of HBV-HCCs, and 13% of HCV-HCCs [83–85]. Notable gain of function (GOF) mutations in HCC include *TP53* V157F and TP53 R249S, the latter being associated with aflatoxin and hepatitis B exposure [83, 86, 87].

Clinically, mutations in the *TP53* family (including p63 and p73), activator p14ARF and inhibitors MDM2 and MDM4, are associated with higher expression of stem cell-like markers, high Edmonson grade, high rates of recurrence, lower disease-free survival, and therapy resistance [19–22]. *TP53* may also downregulate the immune response [23], making these HCCs a potential target for immunotherapy [25]. Therapies targeting *TP53* are aimed at supplementing wild-type p53 or blocking its interaction with cytoplasmic partners. Palbociclib, an oral cyclin-dependent kinase 4/6 inhibitor, has been shown to inhibit p53 DNA-damage partner ataxia telangiectasia mutated (*ATM*) and to increase radiosensitivity of HCC cell lines, with the potential implication that gain-of-function p53 expression can be suppressed [26]. Furthermore, recombinant adenovirus p53, when combined with transarterial chemoembolization (TACE), has been shown to increase overall survival (OS) and disease-free survival (DFS) in patients with HCC [88].

The tumor suppressors ARID1A and ARID1B are components of SWI/SNF complexes that allow DNA repair machinery to access chromatin. Loss-of-function mutations in these genes have historically been associated with alcoholic liver disease and HBV infection [89, 90], and recent studies suggest *ARID1* may contribute to the development of hepatic steatosis [24, 91]. *ARID* mutations typically occur in the later stages of HCC development and result in larger, more aggressive tumors [92–94] with high tumor mutational burdens and increased angiogenesis, both of which potentially make them susceptible to immunotherapy and anti-angiogenic therapies [27, 95].

Telomerases

Telomeres are repetitive nucleotide sequences that provide a docking location for the DNA polymerase complex during replication and also protect chromosomes from deterioration or fusion. When telomeres shorten beyond a critical length after successive rounds of DNA replication, the telomerase complex, composed of a core catalytic telomerase reverse transcriptase (TERT) and RNA template telomerase RNA component (TERC), is activated to lengthen telomeres and restore the liver's regenerative capacity. The TERT promoter mutation is one of the most common genetic alterations in HCC, with an overall frequency of 30–60% [28]. Its presence in low-grade and high dysplastic nodules reveal its role in the early stages of hepatocarcinogenesis [29, 30], which is to drive malignant transformation by selecting for those HCC precursors that escape apoptosis with indefinite telomerase

activity. Ironically, loss of function of telomerase gene variants also predisposes hepatocytes to malignant transformation by impairing hepatocyte response to chronic injury and accelerating cirrhosis [31].

Clinically, TERT promoter mutations are associated with shorter DFS and late intrahepatic recurrence after surgical resection [96]. They are also more frequent in patients with older age, African or European ancestry, and HCV-HCC [32–34]. Other clinically meaningful TERT gene alterations include HBV viral integrations at the TERT gene promoter locus and TERT gene amplifications [96], both of which are associated with decreased OS [97, 98]. The therapeutic promises of TERT mutations are yet to be realized. There are currently no approved therapies targeting TERT mutations, although a phase I clinical trial with an immunotherapeutic agent against hTERT in solid tumors is currently in progress [38]. TERT mutations in circulating DNA may also be a novel way of screening for patients at high-risk patients for HCC [39, 99].

Wnt/β-Catenin Pathway

The Wnt/β-catenin pathway, which in normal tissues are critical for embryonic body axis patterning, cell migration, and cell fate specification, is commonly exploited in hepatocarcinogenesis. *CTNNB1*, which encodes β-catenin, a multifunctional protein that links the intracellular actin cytoskeleton to adherens junctions and also serves as a key nuclear effector of canonical Wnt signaling [100], is mutated in 20–40% of all HCCs [35, 40]. Missense mutations in *CTNNB1* result in higher nuclear and cytoplasmic β-catenin expression in HCCs compared to normal liver, para-carcinoma tissue, and cirrhotic liver [36]. Nuclear expression in HCCs has been associated with more aggressive histopathologic features, such as micro- and macrovascular invasion, increased pathologic grade, increased tumor size, multifocal disease, and tumor recurrence [33, 37]. Furthermore, mutations in any of the proteins responsible for the activation or destruction of β-catenin can lead to aberrant nuclear accumulation. For example, loss-of-function mutations in the *APC*, *AXIN1*, and *AXIN2* genes result in the sustained activation of the Wnt pathway by disruption of the multiprotein destruction complex that tags β-catenin for degradation. Other recently identified members of the Wnt/β-catenin pathway upregulated in HCC include protein regulator of cytokinesis 1 (*PRC1*), *AKIP1*, and thioredoxin protein *TXNDC12* [41, 42, 101]. Germline mutations in the microRNA processing gene *DICER1* have also been associated with *CTNNB1* mutations in familial HCC, although the mechanistic relationship remains unclear [102]. So far, Wnt pathway proteins and genes have not proved to be druggable targets. An antibody that currently targets DKK1, a protein regulator of the Wnt pathway, is currently in phase I clinical trials for HCCs [103].

Other Notable Mutations

Several other notable pathways have been implicated in hepatocarcinogenesis. Mutations in RPS6KA3, a MAP/ERK pathway kinase that was recently shown to be mutated in 4–10% of HCCs, were associated with poor differentiation, macrovascular invasion, high proliferation, and chromosomal instability [104–106]. Genome-wide screening revealed mutations in KEAP1, a master regulator and ubiquitinator of antioxidant gene *NFR2*, to be the top cause of acquired resistance to sorafenib, lenvatinib, and regorafenib in HCC cell lines [107]. HNF1A is a liver-enriched transcription factor (TF) that regulates cellular homeostasis and metabolism. Inactivated or mutated *HNF1A* has been found in HCCs in patients with negative viral status, female sex, and no cirrhosis [43, 44, 108]. A specific point mutation (c.A1532 > T/p.Q511L) causes reduced expression, proliferation, migration, and invasion in HCC cells, while forced expression induces differentiation of these cells into mature hepatocytes [109]. Additionally, the combinatorial transduction of TFs NF4A, HNF1A, and FOXA3 was shown to suppress cellular proliferation of HCC cells [110]**.** Lastly, the transmembrane receptor Janus kinases (JAKs) and the signal transducers STATs are commonly deregulated in HCC [111]. *STAT3* mutations promote a number of cancer hallmarks, such as proliferation, angiogenesis, and metastasis [45–48, 112]. Several small molecule STAT inhibitors, including Stattic, OPB-111077, OPB-31121, Napabucasin, and AZD9150 are currently in preclinical or phase I clinical trials for HCC [49–53].

Thus, recent NGS-based studies have allowed a comprehensive understanding of the somatic mutation landscape of HCC. Although none of the major mutations are directly druggable at present, there are several promising candidates in the pipeline.

Copy Number Alterations

Somatic copy number alterations (SCNAs) result from the gain or loss of individual genes, or more commonly, entire chromosomal arms. The molecular consequence of SCNAs is the potential activation of oncogenes and loss of tumor suppressors, both of which drive carcinogenesis. Multiple studies have shown that copy number gains in chromosomes 1q and 8q, and losses in 8p and 17p, are the most frequent chromosomal arm level alterations in HCC [104, 113, 114]. Apart from the arm-level changes, gene-level changes are also important to identify. The well-known driver oncogenes *CCND1*, *FGF19*, *MYC*, *MET*, *VEGFA*, *MCL1*, and *TERT* were recently shown to be significantly amplified in HCCs [104]. Amplification of *MYC*, a transcription factor known to regulate all of the programs that are hallmarks of cancer, is thought to be an early genomic event in liver

carcinogenesis. It has been found in both chronic liver disease, and in 70% of viral and alcohol-related HCCs [115]. *MYC* amplification at 8q24.1 has been repeatedly associated with large undifferentiated liver tumors, poor prognosis, metastasis, and HCC recurrence [116–118]. Other well-known CNV amplifications include *RB*-regulated transcription factors E2F1 and E2F3. Amplification of these genes resulted in spontaneous HCC in murine models, and queries of the Cancer Genome Atlas (TCGA) datasets revealed a significant increase in the *E2F* family gene dosage in tumors of patients with advanced HCC [119]. Furthermore, copy number increases in matrix metalloproteinase-9 (*MMP9*), which promotes tumor metastasis via the breakdown of the extracellular matrix, were also shown to be associated with key clinicopathological features of HCC such as alpha-fetoprotein (AFP) level, tumor size, differentiation, invasion, and stage [120]. The selective presence of *MMP9* CNVs in tumor tissue over normal tissue makes it a potentially promising diagnostic biomarker for HCC. On the other hand, analysis of HCC tumors has also revealed significant gene deletions in *CDKN2A* and tumor suppressors like *ERRFI1*, *NCOR1*, and *RB1 [121]*, the latter of which is a common mechanism for the development of HBV and HCV HCCs [122].

Other recently identified clinically significant CNVs include *UBE2Q1*, *EXT1*, *WNK2*, *and JAGGED1*. *UBE2Q1* is an E2 ubiquitin-conjugating enzyme thought to promote carcinogenesis via the β-catenin/EGFR-PI3K-Akt-mTOR signaling pathway. Copy number gains in this gene are associated with poorer OS and DS [123]. *EXT1*, which encodes an endoplasmic reticulum glycosyltransferase, has previously been shown to prognosticate breast cancer, cholangiocarcinoma, and acute lymphoblastic leukemia (ALL). *EXT1* mRNA was recently shown to be correlated with serum AFP, and its upregulation was found to be associated with worse DFS [124]. Analysis of 736 primary HCC samples revealed copy number loss of *WNK2*, a potential tumor suppressor, to be associated with early tumor recurrence, macrophage infiltration, tumor growth, and metastasis, likely via *ERK1/2* signaling activation [125]. Amplifications of *JAGGED1*, which encodes a NOTCH pathway ligand, were also recently shown to be associated with poor OS and early HCC recurrence. Lastly, copy number mutations can also have positive therapeutic consequences. Increased *FGF19* copy numbers were associated with a complete response after sorafenib treatment [126].

Epigenetic Modifications

Epigenetic changes, which can occur via DNA methylation, histone modification, chromatin remodeling, and non-coding RNAs, alter the way that genetic code is expressed, rather than directly affecting the nucleotide sequence. Dysregulated DNA methylation has been shown to be an important early event in the pathogenesis of HCC. Studies have noted greater global hypomethylation in HCC tumor tissue, particularly CpG dinucleotides within CpG islands, compared to adjacent tissue, with anywhere from 500 to 684 CpG sites being significantly hypermethylated in matched HCC and normal adjacent tissue comparisons [127, 128]. A 2012 study suggested that these hypermethylated genes may be good early biomarkers for HCC, and five randomly selected genes (*CDKL2*, *STEAP4*, *HIST1H3G*, *CDKN2A*, and *ZNF154*) from the top 18 hypermethylated genes in their study were detectable in the plasma of 63% of patients [127]. A more recent study identified 6 hypermethylated genes (*NEBL*, three *FAM55C* sites, *GALNT3*, and *DSE*) from 375 HCC samples that, when used as biomarkers for HCC, achieved a 98% specificity for HCC [54]. Other individual genes historically found to be hypermethylated in HCC include *APC* (81.7%), *GTP1* (33.3%), *RASSF1a* (66.7%), *p16* (48.3%), *COX2* (35.0%), and Cadherin-1 (*CDH1*) (33.3%) [55, 127]. A meta-analysis of 12 relevant HCC studies covering 981 patients showed that *CDH1* hypermethylation was significantly higher in HCC tissues compared to normal liver and was correlated with worse OS [56].

Epigenetic changes in HCC can potentially be targeted using small molecule inhibitors of DNA methytransferases (DNMTs), which have historically been used in the treatment of myelodysplastic syndrome. Several first-generation DNMTs like azacitidine and decitabine have been shown to reduce tumor formation by inducing hepatic cell differentiation and increasing cell sensitivity to sorafenib in preclinical studies, with decitabine phase I/II clinical trials revealing acceptable safety and toxicity [57, 58]. Second-generation DNMTs like guadecitabine and zebularine were created to improve upon the short half-lives of first-generation DNMTs and are also being tested in phase I/II clinical trials [59].

Non-coding RNAs, which include microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), constitute a well-studied class of epigenetic regulators in HCC. Upregulated expression of mIR-21, mIR-221/222, and mIR-224 have been associated with increased HCC proliferation and migration, while decreased expression of mIR-26, mIR-122, and mIR-199 have been shown to suppress HCC proliferation and angiogenesis [60]. The downregulated expression of mIR 200a was also recently shown to inhibit cell growth, migration, and invasion [61]. These miRNAs may prove to be promising therapeutic targets. Anti-mIR-221 is currently in pre-clinical trials, and treatment with miravirsen, a mIR 122 inhibitor that has completed phase IIa trials, resulted in a substantial and prolonged decrease in plasma mIR-122 in patients with HCV [62, 65]. Notable upregulated lncRNAs include HULC and HOTAIR [63, 64]. HULC is thought to promote HCC proliferation and carcinogenesis indirectly by activating the CREB transcription factor and is also associated with the epithelial-to-mesenchymal transition and angiogenesis [63].

HOTAIR, on the other hand, is thought to maintain the tumor microenvironment via CCL2 expression [64], and its loss was shown to sensitize HCC cells lines to chemotherapy [63, 129].

Histones are protein octamers that help to condense DNA. Modifications to the histone tails that protrude from the DNA/histone nucleosome structure play an important role in the regulation of gene transcription and expression. The placement and removal of acetyl groups from these histone tails by histone acetyltransferases (HATs) and histone deacetylases (HDACs) are often dysregulated in HCC. Even though increased expression of histone deacetylase 3 (HDAC3) has repeatedly been shown to promote HCC proliferation and predict HCC recurrence, deficiency of HDAC3 was recently shown to promote HCC carcinogenesis in a murine model via a defect in the H3K9ac/H3K9me3 transition [130, 131]. Upregulated HDACs 1 and 2 have also been shown to predict mortality in patients with HCC, and they may regulate doxorubicin sensitivity in HCC cell lines [132]. Although they have been used in the treatment of hematological malignancies, HDAC inhibitors have unknown efficacy in HCC. Panobinostat is currently in the preclinical phase of investigation, whereas Belinostat, Resminostat, and CUDC-101 are currently in phase I/II clinical trials [66, 67, 133, 134].

Gene Signatures

Transcriptomic studies in HCC have helped identify gene signatures, or clusters of differentially expressed genes, that can diagnose and prognosticate HCC, and also predict therapeutic response. Historically, gene signatures in HCC have focused on hepatocyte proliferation gene clusters and the EPCAM-positive hepatic cancer stem cell (hCSC) gene clusters. The proliferation cluster, which is expressed across a broad spectrum of human malignancies, includes the A- and B-type cyclins that control the cell cycle at G1/S and/or G2/M transition (*CCNA2*, *CCNB2*), cell division cycle proteins (*CDC2*, *CDC7*, *CDC14*, *CDC20*), heterohexamer DNA helicase minichromosome maintenance protein complex (*MCM3–7*), proliferating cell nuclear antigen (*PCNA*), and DNA topoisomerase 2 α (TOP2A), among others [135, 136]. HCCs that express this gene signature, which largely mirrors a c-MYC-regulated gene signature, are associated with poorer OS and were more likely to also have decreased expression of liver-specific genes that promoted hepatocyte dedifferentiation.

The hCSC gene cluster, on the other hand, was more likely to have increased expression of cell adhesion molecule EpCAM, epithelial marker CK19, and AFP and has clinically been associated with chemotherapeutic resistance [137, 138]. A recent study determined that EpCAM-regulated intramembrane proteolysis helps to drive the hCSC signature and can potentially be targeted for inhibition in HBV-HCCs [68]. The molecular consequence of an hCSC gene signature is de-differentiation of tissues and loss of epithelial morphology, both of which promote malignant tumor behaviors and predict a worse prognosis for patients with HCC [69]. Moreover, hCSC signatures may also be correlated with chemokine networks thought to create a hospitable inflammatory niche for tumor progression and metastasis [70]. A recent study identified an eight gene signature (*TK1*, *CTTN*, *CEP72*, *TRIP13*, *FTH1*, *FLAD1*, *CHRM2*, *AMBP*) in HBV-HCC tumors similar to that found in a previous study that is controlled by transcription factor OCT4, which is abundantly expressed in pluripotent stem cells [71].

Several clinically meaningful gene signatures have recently been identified. Through the application of two different algorithms to screen for differentially expressed genes in paired and unpaired HCC, Zhang et al. identified a 14-gene signature in the cell cycle-related gene cluster (*BIRC5*, *BUB1B*, *CDC45*, *DTL*, *GINS2*, *KIF23*, *KIF2C*, *MAD2L1*, *MCM4*, *OIP5*, *PLK4*, *PTTG1*, and *ZWINT*) that predicts poor OS and HCC recurrence [72]. Another study revealed evidence of network reprogramming in carbon metabolism and cancer pathway genes through the identification of a 22-carbon metabolism gene signature that predicts poorer OS and DFS [73]. Li et al. identified a DNA repair-related prognostic signature of seven genes (*ADA*, *FEN1*, *POLR2G*, *SAC3D1*, *UPF3B*, *SF3A3*, and *SEC61A1)* that, when used to stratify patients into high-risk and low-risk groups, predicts survival in HCC [74]. None of the gene signatures have yet been validated or approved for clinical use, but significant progress is being made towards this goal.

Proteomics

Genetic, epigenetic, and post-translational dysregulation in HCC ultimately results in changes in protein expression levels and protein-protein interactions. Early proteomic studies in HCC, while useful in identifying potential protein targets related to early HCC diagnosis, were limited by smaller sample sizes, lack of validation, and absence of additional functional characterization [75]. These limitations have largely been addressed by advances in high-throughput protein analysis techniques and have resulted in detailed proteomic maps of HCC. Recently, a proteomic and phosphoproteomic comparison between 110 paired HBV-HCC and non-tumor tissues revealed enrichment of cell cycle, integrin, PDGF signaling, MAPK, TNF, and MET pathways, as well as hyperphosphorylation of the p38, RHO, myosin, RB1, and IL1 pathways [139]. Metabolic reprogramming was found to be a key feature of HBV-HCC 1 on paired tumor and adjacent non-tumor liver tissues of 316 patients [140]. Forty-two proteins known to play a role in amino acid metabolism and oxidoreductase activity were dysregulated in HCC. Importantly, the study found that with the exception of a few key metabolic enzymes (SOAT1, SOAT2, GLS, GLUD2), most proteins in liver-specific

pathways, including gluconeogenesis, detoxification, and ureagenesis-ammonia, were significantly decreased in tumors.

Many other proteomic studies are focused on identifying protein targets for either diagnostic or therapeutic purposes. Using an absolute quantitation-based multidimensional liquid chromatography-tandem mass spectrometry technique, Liu et al. identified 27 differentially abundant proteins, mostly in the ERK1/2 and nuclear factor-? beta (NF-KB) pathways, in the serum of patients post-radical resection that were associated with HCC early recurrence [141]. PGK1, a glycolysis enzyme that has been detected in the serum of patients with a broad spectrum of malignancies [76], was specifically identified as an independent predictor of HCC recurrence and OS. Zhao et al. used a high-throughput urinary proteome analysis platform to compare the urine of 74 HCC and 82 high-risk patients with HBV-HCC to identify seven features that distinguish HCC from the high-risk control population in a non-invasive fashion [77]. Thus, large-scale proteomics are adding to our understanding of the functional pathways activated in HCCs and are identifying promising diagnostic and predictive biomarkers for HCC.

Conclusions

HCC is a heterogeneous disease with such a complex genomic landscape. Understanding the molecular drivers of HCC carcinogenesis is essential both to identify biomarkers and to develop molecular targeted therapies. Promising new developments in this field is enabling us to develop therapies that can target the various drivers of HCC and evolve personalized therapeutic strategies. Future genomic studies promise to advance our understanding of this malignancy in meaningful ways and will hopefully ultimately lead to improvement in clinical outcomes for patients with HCC.

Compliance with Ethical Standards

Conflict of Interest No conflicts of interest.

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