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REVIEW

Genetic alterations in hepatocellular carcinoma: An update

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Abstract

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide. Although recent advances in therapeutic approaches for treating HCC have improved the prognoses of patients with HCC, this cancer is still associated with a poor survival rate mainly due to late diagnosis. Therefore, a diagnosis must be made sufficiently early to perform curative and effective treatments. There is a need for a deeper understanding of the molecular mechanisms underlying the initiation and progression of HCC because these mechanisms are critical for making early diagnoses and developing novel therapeutic strategies. Over the past decade, much progress has been made in elucidating the molecular mechanisms underlying hepatocarcinogenesis. In particular, recent advances in next-generation sequencing technologies have revealed numerous genetic alterations, including recurrently mutated genes and dysregulated signaling pathways in HCC. A better understanding of the genetic alterations in HCC could contribute to identifying potential driver mutations and discovering novel therapeutic targets in the future. In this article, we summarize the current advances in research on the genetic alterations, including genomic instability, single-nucleotide polymorphisms, somatic mutations and deregulated signaling pathways, implicated in the initiation and progression of HCC. We also attempt to elucidate some of the genetic mechanisms that contribute to making early diagnoses of and developing molecularly targeted therapies for HCC.

Key words: Genetic alterations; Chromosomal instability; Somatic mutations; Signaling pathways; Hepatocellular carcinoma

Core tip: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide. The poor survival rate is mainly due to late diagnosis of

HCC. Elucidating the molecular mechanisms underlying hepatocarcinogenesis is critical for making early diagnoses of and developing targeted therapies for HCC. Recent studies on HCC using deep sequencing have provided increasing lines of evidence indicating that genetic alterations play important roles in the initiation and progression of HCC, which are summarized in this article. We also attempt to elucidate some of the genetic mechanisms underlying HCC, which may help in making early diagnoses of and developing molecularly targeted therapies for this disease.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third leading cause of cancer-related deaths[1]. HCC has a high incidence rate, and patients with this disease have a poor prognosis. Rising incidence and mortality rates for HCC have been observed in most countries, particularly in eastern/south-eastern Asia and in Africa[2]. Currently, it is generally accepted that persistent hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the primary causes of chronic liver disease leading to liver cirrhosis and HCC. Aflatoxin B1 (AFB1) exposure and chronic alcohol abuse are also important risk factors for developing HCC[2]. Despite improved overall survival (OS) rates among patients with HCC due to advancements in surgical techniques, 5-year OS remains low at 18%[3]. The survival rate of HCC patients is poor because most patients cannot be treated by surgical resections or liver transplantation (LT), mainly due to late diagnosis. In addition, HCC is associated with a high recurrence rate, which exceeds 50% at 5 years after surgery[4]. Therefore, the early detection of HCC is urgently needed to perform curative and effective treatments and to improve long-term survival rates. There is a need for a deeper understanding of the molecular mechanisms underlying the initiation and progression of HCC because this understanding is critical to making early diagnoses and developing novel therapeutic strategies.

It is widely accepted that carcinogenesis is a multistep process triggered by the accumulation of genetic alterations that activate different signal transduction pathways and drive the progressive transformation of normal cells into malignant cells[5,6]. The precise molecular mechanisms underlying the initiation and progression of HCC remain obscure. The phenotypic (morphological and microscopic) and genetic heterogeneity of HCCs also adds a new level of complexity to our understanding of hepatocarcinogenesis. However, despite many remaining challenges, substantial progress has been made in this field. As in other solid cancers, numerous genetic alterations accumulate during the process of hepatocarcinogenesis. Genetic alterations accumulate slowly in a limited number of genes and chromosomal loci during the early preneoplastic stage and accelerate throughout dysplasia and into the development of HCC[7]. Previous studies have shown that the incidence of genetic alterations in HCC is relatively rare and limited to a subset of a few cancer-specific genes[8]. Encouragingly, functional genomic approaches that have been applied in recent years, such as array-based comparative genomic hybridization, genome-wide association studies (GWAS) and next-generation sequencing (NGS), have advanced our understanding of the genetic basis of HCC. Specifically, recent advances in NGS technologies have identified major cancer-driving genes and associated oncogenic signaling pathways that play important roles in the initiation and progression of HCC.

It is known that HCC cells are extremely resistant to almost all conventional chemotherapeutic drugs, and until now, there have been only a limited number of chemotherapeutic agents available for the treatment of patients with HCC, especially those with advanced, unresectable cancer. Currently, oncologists are testing novel, molecularly targeted agents for treating HCC. Therefore, in an era of precision cancer medicine, monitoring clinically relevant genetic alterations is important for stratifying patients for targeted therapies[9].

The molecular mechanisms leading to the development of HCC are extremely complicated and consist of prominent genetic and epigenetic alterations[10]. Although it has been widely accepted that epigenetic alterations also play a significant role in hepatocarcinogenesis, this topic is beyond the scope of this article. Instead, in this article, we focus on the current advances in understanding the genetic alterations, including genomic instability, single-nucleotide polymorphisms (SNPs), somatic mutations, and the deregulated signaling pathways implicated in the initiation and progression of HCC. We also attempt to elucidate some of the underlying genetic mechanisms, which could contribute to making early diagnoses of and developing molecularly targeted therapies for HCC. The impact of genetic alterations on hepatocarcinogenesis is presented in Figure 1.

GENOMIC INSTABILITY

Genomic instability (also known as "genetic instability" or "genome instability") is defined as a high frequency of mutations within the genome, including changes in nucleic acid sequences, chromosomal rearrangements, or aneuploidy[11]. However, it remains unclear whether genomic instability is a cause or a consequence of

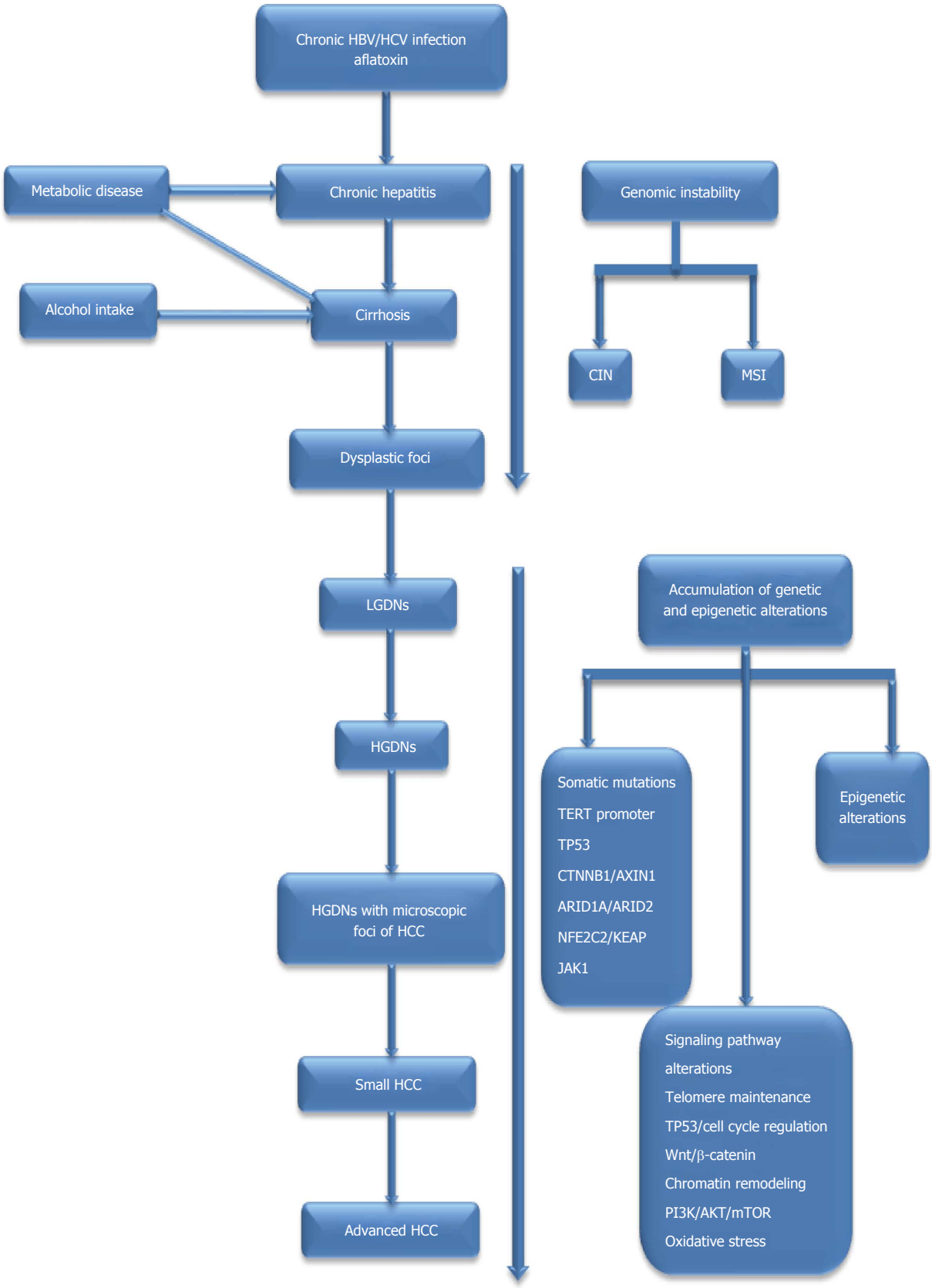

Figure 1 The impact of genetic alterations on hepatocarcinogenesis. Genetic alterations in hepatocarcinogenesis are connected to underlying etiologies, such as HBV, HCV, dietary AFB1 exposure and alcohol intake. Genomic instability accumulates slowly in a limited number of genes during the early preneoplastic stage, such as the development of cirrhosis, and the accumulation of genetic and epigenetic alterations accelerates throughout the formation of preneoplastic lesions, such as LGDNs and HGDNs, and into the development HCC; HBV: Hepatitis B virus; HCV: Hepatitis C virus; AFB1: Aflatoxin B1; LGDN: Low grade dysplastic nodule; HGDN: High grade dysplastic nodule; HCC: Hepatocellular carcinoma; CIN: Chromosomal instability; MSI: Microsatellite instability; TERT: Telomerase reverse-transcriptase; ARID1A: AT-rich interactive domain-containing protein 1A; ARID2: AT-rich interactive domain-containing protein 2; NFE2L2 or NRF2: Nuclear factor erythroid-derived 2-like 2; KEAP1: Kelch-like ECH-associated protein 1; JAK1: Janus kinase 1; RPS6KA3: Ribosomal protein S6 kinase polypeptide 3.

Table 1 The characteristics of chromosomal instability and possible correlations with clinical and pathological parameters in hepatocellular carcinoma discussed in this review

Chromosome	Type of aberration	Targeted genes	Correlations with clinical and pathological parameters	Ref.
1q21	Gain	*CHD1L, CKS1B, JTB, SHC1*	Progression of HCC	Hyeon *et al*[43]
1q21-23	Gain	-	Early development	Yim *et al*[40]
1q21-q22	Gain	-	Metastasis	Wang *et al*[41]
1q21.1-q23.2	Gain	*BCL9, ARNT, TPM3, MUC1, NTRK1*	Poorly differentiated HCV-associated HCC	Liu *et al*[42]
1q22-23.1	Gain	*CD1d*	Diagnosis and prognosis	Zhang *et al*[44]
1q24.1-24.2	Gain	*MPZL1*	Intrahepatic metastasis	Jia *et al*[45]
8q24.21-24.22	Gain	*MYC, DDEF1, MLZE*	Prognosis (DFS and OS)	Pedica *et al*[47]
8q21.13	Gain	*HEY1*	Proliferation	Jia *et al*[37]
8q22.3	Gain	*CTHRC1*	Aggressive HCC	Tameda *et al*[48]
8q24.3	Gain	*BOP1*	Advanced-stage HCC, microvascular invasion and shorter DFS	Chung *et al*[50]
7q21.3	Gain	*SGCE, DYNC1I1, PEG10*	Hepatocarcinogenesis	Tsuji *et al*[51]
4q34.3-35	LOH	*ING2*	Progression	Zhang *et al*[56]
4q13.3-q35.2	LOH	*ADH4, ADH1C, ADH1A, ADH6*	HBV- and AFB1-related HCC carcinogenesis	Qi *et al*[58]
8p	LOH	*DLC1, CCDC25, ELP3, PROSC, SH2D4A, SORBS3*	Early stage of hepatocarcinogenesis, poor outcomes	Tornillo *et al*[59]; Roessler *et al*[30]
8p22-p23	LOH	*MCPH1, KIAA1456, TUSC3, ZDHHC2*	Metastasis and prognosis	Peng *et al*[62]
D4S2964	LOH	*ARD1B, SEPT11*	Prognosis (OS)	Huang *et al*[63]
6q26-q27	LOH	*M6P/IGF2R*	Poor outcomes	Jang *et al*[64]

HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; AFB1: Aflatoxin B1; DFS: Disease-free survival; OS: Overall survival; CHD1L: Chromodomain helicase/ATPase DNA binding protein 1-like; CKS1B: Cyclin-dependent kinases regulatory subunit 1; JTB: Jumping translocation breakpoint; SHC1: SHC-transforming protein 1; BCL9: B-cell CLL/lymphoma 9 protein; ARNT: Aryl hydrocarbon receptor nuclear translocator; TPM3: Tropomyosin alpha-3 chain; MUC1: Mucin 1; NTRK1: Neurotrophic tyrosine kinase receptor type 1; CD1d: Antigen-presenting glycoprotein; MPZL1: Myelin protein zero-like protein 1; MYC: Myelocytomatosis viral oncogene; DDEF1: Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 1; MLZE: Human melanomaderived leucine zipper extra-nuclear factor; HEY1: YRPW motif protein 1; CTHRC1: Collagen triple helix repeat containing 1; BOP1: Block of proliferation 1; SGCE: Epsilon-sarcoglycan; DYNC1I1: Cytoplasmic dynein 1 intermediate chain 1; PEG10: Parternal express gene 10; ING2: Interferon regulatory factor 2; ADH4: Alcohol dehydrogenase 4; ADH1C: Alcohol dehydrogenase 1C; ADH1A: Alcohol dehydrogenase 1A; ADH6: Alcohol dehydrogenase 6; DLC1: Deleted in liver cancer 1; CCDC25: Coiled-coil domain-containing protein 25; ELP3: Longator complex protein 3P; ROSC: Proline synthetase co-transcribed bacterial homolog; SH2D4A: SH2 domain-containing protein 4A; SORBS3: Sorbin and SH3 domain containing 3; MCPH1: Microcephalin 1; KIAA1456: tRNA methyltransferase 9-like; TUSC3: Tumor suppressor candidate 3; ZDHHC2: DHHC-type containing 2; ARD1B: ARD1 homolog B (*S. cerevisiae*); SEPT11: Mus musculus septin 11; M6P/IGF2R: Mannose 6-phosphate/insulin-like growth factor 2 receptor.

tumorigenesis. In recent years, accumulating evidence has strongly indicated that genomic instability could be a major driving force in tumorigenesis and the development of cancer[12-18]. In neoplasms, genomic instability can be broadly classified based on its origin as chromosomal instability (CIN) or, less commonly, microsatellite instability (MSI)[19]. Currently, there are many technologies that can be used to detect genomic instability, including karyotyping, flow cytometry, fluorescent *in situ* hybridization (FISH), array comparative genome hybridization (aCGH), high-density single-nucleotide polymorphism (SNP) arrays, the random amplified polymorphic DNA (RAPD) technique, and NGS technology.

Chromosomal instability

In cancer, aneuploidy is a consequence of an increased rate of whole-chromosome missegregation during mitosis, a process known as chromosomal instability (CIN)[20]. CIN usually involves both numerical and structural chromosomal changes. Numerical CIN is characterized by gross chromosomal abnormalities, such as the gain or loss of whole chromosomes, leading to an altered DNA copy number (aneuploidy)[21]. Structural CIN might involve only fractions of chromosomes, resulting in the gain or loss of chromosome fragments, translocations, inversions, amplifications, deletions and allelic loss [loss of heterozygosity (LOH)][22]. CIN is a hallmark of human cancer and is believed to contribute to tumorigenesis, tumor progression, and the development of therapy resistance[20]. In addition, it has been widely accepted that CIN is associated with clinical and pathological parameters in solid tumors, and CIN is one of the most frequent abnormalities in HCC. The characteristics of CIN and its possible correlations with clinical and pathological parameters in HCC patients are summarized in Table 1. In addition, we also review the role of micronuclei, which are indicators of CIN, and chromothripsis, which is a new class of complex catastrophic chromosomal rearrangement.

DNA copy number alterations (CNAs) are important subclasses of somatic mutations, with aberrant chromosomal regions of amplifications or deletions commonly associated with overexpressed oncogenes or the loss of tumor suppressor genes (TSGs)[23].

Thus, CNAs can be used as an effective method for identifying driver genes with causal roles in carcinogenesis[24]. Such alterations are related to certain types of cancer, including HCC, and it is possible that the identification of driver genes by means of cancer-specific CNAs could provide new insights for understanding the molecular mechanisms underlying the initiation and progression of HCC. In particular, the elucidation of the molecular roles of CNAs could contribute to developing clinically relevant prognostic and predictive markers and novel therapeutic targets for treating HCC, which might ultimately be used in personalized therapeutics. Currently, CNAs in HCC cells are usually detected *via* conventional methods, such as FISH, comparative genomic hybridization, aCGH and SNP arrays. Lately, NGS technology has been used to detect CNAs in several types of tumors[25-27]. These studies have suggested that NGS has obvious advantages in sensitivity, reliability and accuracy in detecting CNAs relative to the use of aCGH and SNP arrays. However, there is currently only one study that has reported NGS-based CNAs detected in HCC[28].

Although the distribution of aberrant chromosomal arms differs among HCCs, numerous studies have shown, using aCGH data and SNP arrays, that certain regions are frequently affected in HCC, including gains in chromosomes 1q, 5p, 6p, 7q, 8q, 17q, and 20q and losses in 1p, 4q, 6q, 8p, 9p, 13q, 14q, 16p-q, 17p, 21p-q, and 22q[28-33]. These findings reflect a high degree of CIN in HCC[34], contributing to hepatocarcinogenesis. In addition, some of these regions contain CNA-associated oncogenes or TSGs, such asc-myelocytomatosis viral oncogene (*c-myc*) (8q), *cyclin A2* (4q), *cyclin D1* (11q), retinoblastoma 1 (*Rb1*) (13q), axis inhibition protein 1 (*AXIN1*) (16p), *p53* (17p), mannose-6-phosphate receptor (*IGFRII/M6PR*) (6q), *p16* (9p), epithelial cadherin (*E-cadherin*) (16q), suppressor of cytokine signaling (*SOCS*) (16p), and phosphatase and tensin homolog (*PTEN*) (10q), which have been identified to be associated with HCC[35,36]. These findings could provide us with information critical for understanding the genetic events involved in the pathogenesis and progression of HCC. However, studies employing unbiased genome-wide searches for HCC driver genes have been limited, particularly for genes related to cancer prognosis[30]. Hence, an integrated approach, such as a combined analysis of CNAs and gene expression, might be necessary to identify driver mutations.

A copy number gain at 1q is one of the most frequently detected alterations in HCC (58%-86%), and it has been suggested to be an early genomic event in the development of HCC[37]. Notably, the region 1q21 is the most frequent minimal amplifying region (MAR)[38]. A research group showed that 1q21 was the most frequently amplified region in chromosome 1q; its amplification was detected in 36 of 60 (60%) HCC specimens[39]. In addition, a gain in 1q21-23 was identified as a genomic event associated with the early development of HCC[40], and regional 1q21-q22 gains were found in 40% of advanced metastatic HCC cases[41]. In particular, a gain of 1q21.1-q23.2 was significantly associated with grades Ⅱ-Ⅳ HCC and moderately or poorly differentiated HCV-associated HCCs. 1q21.1-q23.2 target genes encode five cancer genes: B-cell CLL/lymphoma 9 protein (*BCL9*), aryl hydrocarbon receptor nuclear translocator (*ARNT*), tropomyosin alpha-3 chain (*TPM3*), mucin 1 (*MUC1*), and neurotrophic tyrosine kinase receptor type 1 (*NTRK1*)[42]. These findings indicate that 1q21 might harbor many potential oncogenes, and the overexpression of these genes *via* amplification plays an important role in the pathogenesis of HCC[38]. In recent years, several research groups have focused on the identification and characterization of 1q21 target genes, such as chromodomain helicase/ATPase DNA binding protein 1-like *(CHD1L),* cyclin-dependent kinase regulatory subunit 1 (*CKS1B),* jumping translocation breakpoint (*JTB*) and SHC-transforming protein 1 (*SHC1),* in the progression of HCC. Of these, *CHD1L* was shown to be amplified and overexpressed in HCC cases[39]. A recent study found no nuclear immunoreactivity for *CHD1L* in normal livers or dysplastic nodules (DNs). In contrast, *CHD1L* expression in cases of HCC was significantly associated with microvascular invasion, major portal vein invasion, and higher American Joint Committee on Cancer (AJCC) T stage values[43], suggesting that *CHD1L* expression might not be an early event in hepatocarcinogenesis, whereas it is an independent predictor of lower disease-free survival (DFS) rates in HCC patients after surgical resection. Given these findings, it is vital to elucidate the roles of candidate target genes within 1q21 amplicons in the initiation and progression of HCC, which could contribute to our understanding of HCC carcinogenesis.

In addition to chromosome 1q21, a novel potential oncogene antigen-presenting glycoprotein (*CD1d*) amplicon at 1q22-23.1 could be a potential target for this amplicon in HCC[44]. In addition, using an integrated analysis of copy number and expression profiling data, one recent study found that the recurrent region of the 1q24.1-24.2 amplicon specifically targets the myelin protein zero-like protein 1 (*MPZL1*) gene in HCC; the expression levels of *MPZL1* were positively correlated with the intrahepatic metastasis of the HCC specimens[45].

Chromosome 8q is the second most frequently amplified region in HCC. More specifically, 8q24.21-24.22 is the most frequently amplified region in chromosome 8q, with amplification occurring in 53.4% of samples and targeting the known oncogenes myelocytomatosis viral oncogene (*MYC*), Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 1 (*DDEF1*), and human melanoma-derived leucine zipper extra-nuclear factor (*MLZE*)[45]. *MYC* has been identified as a central regulator of malignant transformations in early hepatocarcinogenesis[46], and

c-myc gene amplification has also been found to be significantly correlated with DFS and OS in patients with HCC after surgical resection[47]. These findings suggest that *c-myc* gene amplification plays an important role in the pathogenesis and progression of HCC. Additionally, three other recurrent amplified regions at chromosome 8q have been found: 8q21.13, 8q22.3, and 8q24.3. The 8q21.13 region targets the hairy/enhancer-of-split related with YRPW motif protein 1 (*HEY1*), and functional experiments have shown that the enhanced expression of *HEY1* significantly promotes the *in vitro* and *in vivo* proliferation of HCC cells[37]. The 8q22.3 region targets two genes: collagen triple helix repeat containing 1 (*CTHRC1*) and grainy head-like transcription factor 2 (*GRHL2*). *CTHRC1* has the potential to be a new biomarker of aggressive HCC[48], while a gain in *GRHL2* was found to be associated with an early recurrence of HCC[49]. The 8q24.3 region contains several genes that could be functionally related to HCC, including scribble (*SCRIB*) and block of proliferation 1 (*BOP1*). It has been reported that increased expression of *BOP1* is associated with advanced-stage HCC, microvascular invasion and lower DFS[50].

Other amplifications include the 7q21.3 locus, which might contribute to the development or progression of HCC. Epsilon-sarcoglycan (*SGCE*), cytoplasmic dynein 1 intermediate chain 1 (*DYNC1I1*) and paternal express gene 10 (*PEG10*) have been identified as putative oncogenes located within the amplified 7q21.3 locus in HCC[51,52]. These results indicate that the amplification of 7q21.3 might be implicated in hepatocarcinogenesis.

The LOH is a marker of CIN that involves the loss of one of the two alleles at one or more loci in a heterozygote[53]. The LOH is one of the main mechanisms for the inactivation of TSGs, and the identification and characterization of LOHs could provide potential means for finding HCC-related TSGs. The LOH is frequently observed on chromosomes 1p, 4q, 6q, 8p, 9p, 10q, 11p, 13q, 14q, 16q, and 17p and is commonly observed in HCC patients[54,55]. Of these, losses on 4q and 8p are the most frequent chromosomal alterations in HCC.

The LOH at 4q has been reported to be strongly correlated with increased in alpha-fetoprotein (AFP) levels in HCC[56], and it has been found to be significantly more frequently in poorly differentiated HCCs[57]. These results suggest that the inactivation of TSGs on chromosome 4q might be a late progression event that occurs after malignant transformation. Using a high-throughput SNP array, 4q24-26 and 4q34.3-35 were found to be hot regions of chromosome 4q in HCC[56]. Three TSGs, including nei endonuclease VIII-like 3 (*NEIL3*), interferon regulatory factor 2 (*IRF2*) and inhibitor of growth family member 2 (*ING2*), are located on chromosome 4q34.3-35, but only *ING2* is a potential TSG associated with HCC[48]. In addition, the loss of 4q13.3-q35.2 is related to both HBV- and AFB1-related HCC[58], suggesting that genetic abnormalities in 4q13.3-q35.2 might play a role in both HBV- and AFB1-related HCC carcinogenesis. Four TSGs, including alcohol dehydrogenase 4 (*ADH4*), alcohol dehydrogenase 1C (*ADH1C*), alcohol dehydrogenase 1A (*ADH1A*), and alcohol dehydrogenase 6 (*ADH6*), are located in this region[58].

The LOH on chromosome 8p is one of the most common alterations in HCC. A group of researchers found that allelic losses on 8p were observed in high-grade dysplastic nodules (HGDNs)[59], indicating that these losses might occur in the early stage of hepatocarcinogenesis. Chromosome 8p is rich in candidate and validated TSGs, with a cluster of six genes, including deleted in liver cancer 1 (*DLC1*), coiled-coil domain-containing protein 25 (*CCDC25*), elongator complex protein 3 (*ELP3*), proline synthetase co-transcribed bacterial homolog (*PROSC*), SH2 domain-containing protein 4A (*SH2D4A*), and sorbin and SH3 domain containing 3 (*SORBS3*), located on chromosome 8p that have been deleted in HCC samples from patients with poor outcomes[30]. Notably, numerous studies have revealed a high frequency for LOH on 8p22-p23 in HCC[60,61], and deletions of alleles on 8p22-p23 have been found to be associated with metastasis and poor prognoses for HCC patients[56]. Four specific genes - microcephalin 1 (*MCPH1*), tRNA methyltransferase 9-like (*KIAA1456*), tumor suppressor candidate 3 (*TUSC3*), and zinc finger, DHHC-type containing 2 (*ZDHHC2*) - are located in this region. Of these genes, a LOH for *ZDHHC2* might contribute to the early metastatic recurrence of HCC after LT[62]. These findings suggest that 8p22-p23 harbors numerous TSGs that play important roles in the progression of HCC, which could contribute to assessing the risk of metastasis and recurrence in HCC patients.

In addition, a few recent studies have investigated the associations between LOH for new TSGs and the clinicopathological features of HCC. For example, LOH in the genes ARD1 homolog B (*S. cerevisiae*) (*ARD1B*) and *Mus musculus* septin 11 (*SEPT11*) were found to be significant prognostic factors for poor OS[63], and LOH in mannose 6-phosphate/insulin-like growth factor 2 receptor (*M6P/IGF2R*) was found to be predictive of poor clinical outcomes in surgically resected primary HCC patients[64].

In summary, the aforementioned findings provide valuable information that could contribute to our understanding of HCC carcinogenesis. However, there are still many important LOH regions that must be explored with regard to the genes that are involved in carcinogenesis and their biological and clinical implications[63].

MN are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that are not incorporated into the nucleus after cell division[65]. The frequency of MN is higher in tumor cells and cells with defective DNA damage repair systems or

disrupted cell cycle checkpoint machinery; hence, MN could serve as indicators of CIN[66,67]. In one study, the micronucleus index was found to gradually increase along with the progression of hepatocarcinogenesis. HCCs showed the highest micronucleus index values, which were significantly greater than those of HGDNs and DNs with HCC foci[68]. In another study, a progressively increasing number of MN were also documented in the transition from cirrhotic nodules (CNs) to large regenerative nodules (LRNs), DNs and HCC; MN were significantly more frequent in DNs than in CNs or LRNs[69]. These results suggest that CIN might occur in the early stage of hepatocarcinogenesis, and HCC cells generally have acquired chromosomal abnormalities; therefore, the degree of CIN could increase during the progression of HCC.

Recently, chromothripsis has been identified using whole-genome sequencing(WGS) as a new class of complex catastrophic chromosomal rearrangement. Chromothripsis is a single cellular crisis in which a chromosome is broken and reassembled by a DNA repair mechanism, resulting in a large number of rearrangements clustered in a chromosomal region[70]. Although chromothripsis appears to be relatively rare, it can be an extreme outcome of a mutagenic mechanism that could be widespread in human cancers[71]. Furthermore, chromothripsis could affect cancer gene function and thereby have a major impact on the progression, prognosis, and therapeutic response of cancer[72]. To date, we are aware of only one study that investigated the role of chromothripsis in the incidence of HCC. In this study, chromothripsis and CIN were found to recurrently affect chromosomal arms 1q and 8q to create gene amplifications, suggesting that chromothripsis might contribute to hepatocarcinogenesis[33]. It seems that more attention should be paid to this concept.

MSI

MSI is the result of defects in mismatch repair genes that leads to the expansion and contraction of short nucleotide repeats called microsatellites[18]. Microsatellites are simple tandem repeats that are present at millions of loci in the human genome. MSI can result in the inactivation of TSGs or can disrupt other noncoding regulatory sequences, thereby playing a role in carcinogenesis[73]. MSI has been described in cirrhosis, mainly when cirrhosis is associated with an HBV infection[74,75]. Recent limited data are available on the incidence of MSI in HCCs. Several studies have suggested that MSI might play a minor role in hepatocarcinogenesis[76,77]. Furthermore, MSI is not implicated in the pathogenesis of a subset of HCCs affecting elderly patients without chronic liver disease[78]. Nevertheless, two studies have shown that high levels of MSI (MSI-H > 30%) were significantly associated with more aggressive histological tumor features and shorter median delays before recurrence[79], and the degree of MSI was significantly correlated with the poor differentiation and portal vein involvement of HCC[80]. These findings suggest that MSI could play a minor role in hepatocarcinogenesis and might be associated with the progression of HCC in patients with a background of chronic hepatitis and/or cirrhosis.

SNPS

SNPs are the most common form of human genetic polymorphisms that can contribute to an individual's susceptibility and progression to cancer. Accumulating evidence suggests an association between SNPs in certain genes and HCC susceptibility[81]. GWAS have emerged as a new approach for identifying less penetrant cancer susceptibility alleles that might be associated with the initiation and progression of cancer.

Recent GWAS have identified numerous SNPs associated with the risk of HCC (Table 2); however, most findings have been both conflicting and inconsistent. For example, three researchers investigated whether an SNP (rs17401966) of kinesin-like factor 1 B (*KIF1B)* might be associated with the risk of HBV-related HCC in Chinese individuals. One study found that it was[82], but another study found that it was not[83]. A third study found that *KIF1B* alone was not associated with the risk but that the gene-environment interaction between the *KIF1B* variant and alcohol consumption was associated with the risk of HCC[84]. These inconsistent findings could be attributed to a lack of controlling for confounding variables, such as epidemiological and environmental risk factors in the first two studies. Therefore, it is important to evaluate the role of *KIF1B* rs17401966 in the genetic susceptibility to HCC and gene-environment interactions. Interestingly, three studies found that *KIF1B* rs17401966 was not associated with the development of HBV-related HCC in Thai, Japanese, and Saudi Arabian patients[85-87], and two other studies identified that *KIF1B* rs17401966 exerted protective effects against the susceptibility to HBV-related HCC in Chinese patients[88,89]. These inconsistencies might partly be because different ethnicities or study populations have distinct genetic architectures. In another example, three GWAS identified that MHC class Ⅰ polypeptide-relatedsequence A (*MICA*) and DEP domain containing 5 (*DEPDC5*) SNPs were strongly associated with HCC in Japanese populations with chronic HCV infections[90-92]. However, two other studies found that neither *DEPDC5* rs1012068 nor *MICA* rs2596542 was associated with HCC in Europeans with chronic HCV infections[93] or in Chinese populations with chronic HBV infections[94]. The discrepancies among these studies might be due to different study designs[93] or to differences in the different racial/ethnic groups. The inconsistent findings for HBV- and HCV-related HCC suggest that whether SNPs in

Table 2 Summary of single-nucleotide polymorphisms associated with the risk of hepatocellular carcinoma identified from genome-wide association studies

Related gene	SNP	Etiology of HCC	Odds ratio (95%CI)	*P* value	Ref.
TPTE2	rs2880301	HBV/HCV, Republic of Korea	0.27 (0.19-0.39)	1.74×10^{-12}	Clifford *et al*[96]
KIF1B	rs17401966	HBV, China	0.61 (0.55-0.67)	1.70×10^{-18}	Zhang *et al*[82]
KIF1B	rs17401966	HBV interacting with alcohol consumption, China	2.36 (1.49-3.74)		Chen *et al*[84]
GRIK1	rs455804	HBV, China	0.84 (0.80-0.89)	5.24×10^{-10}	Li *et al*[94]
HLA-DQA1/DRB1	rs9272015	HBV, China	1.28 (1.22-1.35)	1.13×10^{-19}	Li *et al*[94]
MICA	rs2596542	HCV, Japan	1.39 (1.27-1.52)	4.21×10^{-13}	Kumar *et al*[91]
HLA-DQ	rs9275319	HBV, China	1.51 (1.38-1.66)	8.65×10^{-19}	Jiang *et al*[97]
DEPDC5	rs1012068	HCV, Japan	1.75 (1.51-2.03)	1.27×10^{-13}	Miki *et al*[90]
DDX18	rs2551677	HBV/HCV, Republic of Korea	3.38 (2.07-5.53)	1.41×10^{-10}	Clifford *et al*[96]
FasL	rs763110	HBV/HCV, Egypt	1.970 (1.250-3.105)	0.003	Khalifa *et al*[98]
DLC1	rs3816747	HBV, China	0.486 (0.245-0.962)/ 0.51 (0.267-0.974)	0.037/0.039	Xie *et al*[99]
STAT4	rs7574865	HBV, China	1.22 (1.15-1.29)	1.66×10^{-11}	Jiang *et al*[97]
FOXP3	rs3761549	HBV, China	1.32 (1.03-1.70)	0.030	Chen *et al*[100]

SNP: Single-nucleotide polymorphism; HCC: Hepatocellular carcinoma; GWAS: Genome-wide association studies; HBV: Hepatitis B virus; HCV: Hepatitis C virus; TPTE2: Transmembrane phosphoinositide 3-phosphatase and tensin homolog 2; KIF1B: Kinesin-like factor 1 B; GRIK1: Glutamate receptor, ionotropic, kainate 1; HLA-DQA1/DRB1: Major histocompatibility complex, class II, DQ alpha 1, DR beta 1; MICA: MHC class I polypeptide-relatedsequence A; HLA-DQ: Major histocompatibility complex class II antigen; DEPDC5: DEP domain containing 5; DDX18: DEAD (Asp-Glu-Ala-Asp) box polypeptide 18; FasL: Fas ligand; DLC1: Deleted in liver cancer 1; STAT4: Signal transducer and activator of transcription 4; FOXP3: Forkhead box P3.

the *MICA* and *DEPDC5* loci affect the susceptibility to HCC is subject to race/ethnicity-specific differences. Undoubtedly, the same variability also applies to all the other HCC-related SNPs, which could be explained by gene-gene and gene-environment interactions contributing to the inconsistent findings in different racial or ethnic groups that have been studied[95].

Taken together, the available results show that most findings related to the SNPs detected in GWAS on HCC can be problematic to replicate due to differences among different racial/ethnic groups, different study designs, and genetic heterogeneity. GWAS have so far identified numerous SNPs associated with HCC susceptibility[90-100]; however, most of these investigations were limited by relatively small sample sizes or the inclusion of only one racial/ethnic group. The inconsistency of these findings could be attributed to many factors, such as a lack of control for confounding variables, different study designs or the different racial/ethnic groups in the studies. Given the high variability/inconsistency in findings related to SNPs found in GWAS, at least to date, we cannot recommend the continued study of SNPs in relation to HCC as a means for identifying reliable markers of the initiation and progression of HCC. Therefore, further well-designed investigations with larger sample sizes and multiple races/ethnicities are warranted to elucidate the impact of SNPs on susceptibility to HCC.

SOMATIC MUTATIONS IN HCC

Similar to any other cancer, HCCs consist of highly heterogeneous tumors with multiple genetic alterations, particularly somatic mutations. Recent advances in NGS technologies, such as WGS or whole-exome sequencing (WES), have enabled us to identify global driver genes related to the development of HCC. In addition to confirming the high frequency of somatic mutations in tumor protein p53 (*TP53*), catenin beta 1 (*CTNNB1*) and *AXIN1*, recent studies applying deep-sequencing analyses have identified numerous novel mutations in genes, such as mutations in genes related to chromatin remodeling (*ARID1A* and *ARID2*), oxidative stress (*NFE2L2* and *KEAP1*), RAS/MAPK signaling (*RPS6KA3*), and the janus kinase/signal transducers and activators of the transcription (JAK/STAT) pathway (*JAK1*)[28,101-103]. With the exception of *ARID1A* (10%-16%), most of these newly identified driver genes are mutated in less than 10% of HCC cases. It is encouraging that recurrent telomerase reverse transcriptase (*TERT*)-promoter mutations have been recently identified as the most frequent molecular alterations in HCC and as the first gene that is recurrently mutated in cirrhotic preneoplastic lesions[104,105]. There is abundant evidence to support the notion that *TERT, TP53, CTNNB1, ARID1A* and *AXIN1* are recurrently mutated genes involved in HCC[28,102,103,106-111]. Specifically, driver mutations in *TERT, TP53,* and *CTNNB1* are among the most frequent genetic alterations that have been defined as additive events in the development of HCC, irrespective of etiological background[28,106-108,112-115].

In this section, we briefly summarize previously well-known driver mutations and some novel gene mutations discovered in NGS studies. *CTNNB1* and *AXIN1* are subsequently reviewed in relation to the Wnt/β-catenin signaling pathway. The role and characteristics of frequent recurrent somatic mutations in HCC and their associations with clinical pathological

Table 3 The characteristics of frequent recurrent somatic mutations and their correlations with clinical and pathological parameters in hepatocellular carcinoma based on deep-sequencing analyses

Gene	Altered pathway	Correlations with clinical and pathological parameters	Ref.
TERT promoter	Telomere stability	Hepatocarcinogenesis	Nault *et al*[104]; Yang *et al*[165]
TP53	Cell cycle control	Under debate: an early event in the context of aflatoxin exposure and chronic HBV infection, or it might not play a role in carcinogenesis	Qi *et al*[136]
		Poor prognosis	El-Din *et al*[117]
			Cleary SP *et al*[138]
CTNNB1	Wnt/β-catenin signaling	Under debate: a late event for malignant progression or earlier during hepatocarcinogenesis	Park *et al*[253]; Vilarinho *et al*[256]
		Under debate: worse outcomes or better outcomes	Tornesello *et al*[263]; Wang *et al*[269]
AXIN1	Wnt/β-catenin signaling	Hepatocarcinogenesis and progression	Guan *et al*[242]
ARID1A	Chromatin remodeling	Initiation and progression of HCC	Schulze *et al*[106]
ARID2	Chromatin remodeling	Initiation and progression of HCC	Totoki *et al*[107]
NFE2L2	Oxidative stress	Hepatocarcinogenesis and progression	Nault JC *et al*[105]
KEAP1	Oxidative stress	Hepatocarcinogenesis and progression	Schulze *et al*[106]
JAK1	JAK/STAT pathway	Hepatocarcinogenesis	Kan *et al*[28]
RPS6KA3	RAS/MAPK signaling	Hepatocarcinogenesis	Guichard *et al*[102]

TERT: Telomerase reverse-transcriptase; ARID1A: AT-rich interactive domain-containing protein 1A; ARID2: AT-rich interactive domain-containing protein 2; NFE2L2/NRF2: Nuclear factor erythroid-derived 2-like 2; KEAP1: Kelch-like ECH-associated protein 1; JAK1: Janus kinase 1; RPS6KA3: Ribosomal protein S6 kinase polypeptide 3.

parameters are summarized in Table 3.

TP53

TP53 is a key molecule in the *TP53*/cell cycle signaling pathway. The mutation or deletion of the *p53* gene, which plays an important role in cell growth, division and apoptosis by acting as a transcription factor or by forming complexes with other proteins, is one of the most frequent genetic changes detected in HCC[116,117]. Strikingly, *TP53* mutation rates in HCC vary in different geographic areas, reflecting differences in etiological agents and susceptibility factors[118]. The *TP53* mutation in HCC occurs most commonly in sub-Saharan Africa and Southeast Asia, where the combination of widespread dietary AFB1 exposure and endemic hepatitis B fosters a high rate of mutagenesis in the liver[119]. In these areas, AFB1 is a particularly common mutagen of *TP53*, causing G:C to T:A transversions at the third base of codon 249 in TP53 (R249S), and the rate of *TP53* R249S mutations can be accelerated in the presence of a viral infection[120,121]. This mutation was not detected in HCC cases from non-aflatoxin-contaminated areas[119].

Accumulating evidence shows that the HBV X (HBx) protein is a multifunctional regulator that plays a crucial role in HBV-associated hepatocarcinogenesis[122]. However, the potential synergistic effects between the HBx protein and *TP53* mutations during hepatocarcinogenesis remain unclear. Several studies have suggested that the HBx protein affects the function of the P53 protein and contributes to the development of HCC. For example, complete HBx sequences were often associated with the presence of *TP53* R249S mutations[123], and HBx was found to be associated with *TP53* R249S mutations in HCC patients with no documented history of cirrhosis[124]. In addition, HBx mutations were found to interact with *TP53* R249S mutations in altering cell proliferation and chromosome stability in hepatocytes[125]. HBx has also been shown to bind to *p53* and to block *p53*-sequence-specific DNA-binding and *p53*-dependent transcription, ultimately blocking *p53*-mediated apoptosis[126]. HBx and *TP53* mutations have been suggested to synergistically contribute to the formation of HCC in animal models[127]. These findings suggest that HBx is involved in the etiology of *TP53* mutations during the molecular pathogenesis of HCC.

Persistent HCV infections could play a role in hepatocarcinogenesis; however, the mechanisms underlying this process remain unclear. A possible mechanism of HCV-induced oncogenesis seems to result from the interference of HCV proteins in the intracellular signal transduction processes *via* a mechanism including the dysregulation of cell cycle control[128]. In the presence of DNA damage, the P53 protein can be activated, promoting the expression of several important genes involved in cell cycle arrest, DNA repair, and apoptosis[129]. Accordingly, whether HCV infections occur concurrently with other genomic alterations, such as *TP53* mutations, in hepatocarcinogenesis is of interest. Currently, several studies have provided some evidence for the direct action of HCV-related proteins on *TP53*. For example, HCV infection impairs the function of P53 through the overexpression of 3β-hydroxysterol delta 24-reductase (*DHCR24*), which up-regulates the interaction between P53 and MDM2 (mouse double minute 2 homolog, also known as HDM2, a P53-specific E3 ubiquitin ligase) in the cytoplasm and suppresses P53 acetylation in the nucleus[130]. Additionally, a novel *TP53* mutation, 616ins14del1 (14-1 microindel), has been detected in a case of HCC associated with an HCV infection,

providing evidence that HCCs characterized by HCV infections are typically associated with the mutational inactivation of the *TP53* gene[131]. In addition, genetic changes in *TP53* have been detected in non-neoplastic lesions linked to chronic HCV infections[132]. Collectively, the aforementioned findings suggest that HCV is implicated in the etiology of *TP53* mutations during hepatocarcinogenesis. However, these results were obtained *in vitro* using cell culture models or animal models, and the synergistic effects of *TP53* mutations and HCV infections in human hepatocarcinogenesis must be further investigated.

A *TP53* mutation has been identified as one of the most frequent molecular alterations in HCC; however, the role of *TP53* mutations in hepatocarcinogenesis remains debatable. Strikingly, a missense mutation in exon 7 (R249S) of *p53* has been found specifically in HCC patients from regions with high levels of AFB1 exposure[133]. Several studies have suggested that *TP53* R249S mutations are likely to occur as early events in association with aflatoxin exposure and chronic HBV infection[134-136]. A recent study showed that *TP53* R249S mutations are an important factor in HCC carcinogenesis in Brazil, where aflatoxin exposure levels are high[137]. In contrast, *TP53* mutations can occur as a late event in carcinogenesis without a typical mutational pattern in areas with low levels of AFB1 intake[135]. Furthermore, another study showed that *TP53* R249S mutations might not play a role in the carcinogenesis of HCC in Egypt, where HCV infections are highly prevalent and are a major risk factor for the development of HCC[117]. Taken together, these findings show that *TP53* mutations could play an important role in hepatocarcinogenesis in populations with chronic HBV infections, especially in those exposed to excessive levels of AFB1. It follows that these inconsistent and even conflicting results regarding the role of *TP53* mutations in hepatocarcinogenesis might primarily be due to heterogeneity in the geographic and etiological backgrounds of the cases studied.

Recent reports have shown that *TP53* mutations can be used to predict HCC. For example, mutations in *TP53* were found to be associated with a significantly higher rate of recurrence and a lower DFS[138]. In addition, two systematic reviews concluded that *TP53* mutations were associated with poor OS, relapse-free survival rates (RFS), and DFS in HCC patients, with similar results found between patients with HBV infections and HCV infections[139,140]. However, a recent study showed that *TP53* mutations were associated with shorter survival time only in cases of HBV-related HCC, although R249S hot spot mutations were not associated with survival rates in patients of European origin with HBV-related HCC[141]. In contrast, another study found that *TP53* mutations, particularly the hot spot mutations R249S and V157F, regardless of sample origin, were associated with poor prognoses in patients with HCC[142]. This finding was echoed by another recent study on the relationship between *TP53* mutations and the recurrence of HCC in patients with HCC of various etiologies[143]. Taken together, these inconsistent and even conflicting findings might be largely due to the use of different racial and regional groups as well as other possible contributing factors, including the small sample sizes of the studies. Therefore, these confounding factors should be considered when evaluating the prognostic value of *TP53* mutations in HCC.

Increasing evidence suggests that the stabilization of mutant *p53* in tumors is crucial for its oncogenic activities, while the depletion of mutant *p53* attenuates the malignant properties of cancer cells. Thus, mutant *p53* is an attractive drug target for cancer therapies[144].

Telomerase reverse-transcriptase

The human telomerase reverse transcriptase (*hTERT*) gene encodes a rate-limiting catalytic subunit of telomerase, which maintains the length of telomeric DNA and chromosomal stability[145]. *hTERT* is the major determinant of telomerase activity, and it plays a key role in cellular immortalization and the development and progression of human cancers. The reactivation of telomerase activity is observed in approximately 90% of human cancers, enabling cells to overcome replicative senescence and to escape apoptosis, which are fundamental steps in the initiation of malignant transformation[146,147]. The precise mechanism behind the reactivation of telomerase activity in cancer remains elusive, but it likely involves multiple changes that occur during the progression of cancer, including mutations and chromosomal rearrangements[148].

In two recent studies, researchers identified mutations that created new binding sites in the *TERT* promoter for particular transcriptional regulators, such as E-twenty-six (*ETS*)/ternary complex factors (*TCFs*) factors, and resulted in increased transcriptional activity at the *TERT* promoter, which could in turn lead to the increased expression of the gene and the endless cell division characteristic of cancer cells[149,150]. These findings suggest that *TERT* promoter mutations could be potential mechanisms for *TERT* reactivation in cancer cells. In more recent studies, investigators found that two highly recurrent point mutations (G228T and G250T) in the *TERT* promoter might be among the fundamental mechanisms underlying telomerase reactivation/expression in several types of human cancers[149,151-154].

The molecular mechanisms involved in telomerase reactivation in HCC have been only partially elucidated, with the most important being *TERT* promoter mutations[104]. *TERT* amplification and the recurrent integration of HBx into the *TERT* gene promoter are alternative explanations for telomerase reactivation[107,155-157]. In particular, *TERT* promoter mutations were found to be associated with *CTNNB1* mutations in HCC[104,106,107,158], suggesting that

TERT promoter mutations and the deregulation of the Wnt/β-catenin pathway could interact in the malignant transformation of hepatocytes. Overall, the identification of *TERT* promoter mutations in association with HCC has provided new insights into telomerase reactivation and telomere maintenance in hepatocarcinogenesis[148]. Despite these compelling findings, the functional role of *TERT* promoter mutations in HCC remains unclear and must be further explored.

To date, recurrent somatic mutations in the *TERT* promoter have been identified as the most frequent non-coding mutations in multiple cancer types, suggesting that *TERT* promoter mutations are driver mutations in these cancers[154,159,160]. The frequency of *TERT* promoter mutations in HCC varies substantially across the different geographical regions studied. For example, cases of HCC with *TERT* promoter mutations have been reported from the United States[161], Europe[104,158,162], Africa[163], and East Asia (except for Japan)[103,104,164-166], with mutation frequencies of 44%, 47%-59%, 53%, and 20.7%-38.8%, respectively. These data indicate that *TERT* promoter mutations are less frequent among Asian patients with HBV-related HCC than among those with HCV-related HCC. The lower rate of *TERT* promoter mutations in patients with HBV-related HCC might be partially explained by the frequent insertion of HBV DNA in the *TERT* promoter, which is known to induce telomerase transcription[103,155]. These findings suggest that various etiological factors could be involved in different mechanisms that preserve telomeres during the carcinogenesis of HCC[164]. Despite these differences, *TERT* promoter mutations are currently considered the most frequent somatic genetic alterations in HCC regardless of patients' geographical origin[163,167]. In the past few years, many investigators have explored the role of *TERT* mutations in HCC. In a recent study, *TERT* promoter mutations were found in 6% of low-grade dysplastic nodules (LGDNs), 19% of HGDNs, 61% of early HCCs and 42% of small and progressed HCCs. However, mutations in other classic HCC driver genes (*i.e*., *CTNNB1, TP53, ARID1A*, or *ARID2*) were not identified in LGDNs, HGDNs, or early HCC[105]. In another recent study, *TERT* mutations were found to occur at an early stage of tumorigenesis. Specifically, they were observed in 57% of preneoplastic lesions and in 30% of stage I HCCs[165], indicating that *TERT* promoter mutations occur early during malignant transformation and persist throughout tumor progression. These findings have been further confirmed by two recent studies using exome or DNA sequencing of liver tumor samples in which *TERT* promoter mutations occurred early during hepatocarcinogenesis[106,164]. In addition, when *hTERT mRNA* was measured *via* real-time quantitative RT-PCR, the *hTERT mRNA* levels were found to be increased in association with the progression of hepatocarcinogenesis, and most HGDNs strongly expressed *hTERT mRNA* at levels similar to those in HCC samples[168]. In a recent study, the authors found that the activation and expression of *hTERT* played extremely critical roles in the incidence and progression of HCC[169]. Previous studies also showed that telomere shortening and telomerase reactivation occurred in DNs during the early stages of hepatocarcinogenesis[163]. Indeed, alterations in telomerase restriction fragment (TRF) length, telomerase activity (TA), and *hTERT* and *hTR* expression were identified in both the early and late stages of hepatocarcinogenesis[170]. These findings demonstrate that telomere status is a factor in hepatocarcinogenesis.

hTERT mRNA has been reported to be detectable in the serum of patients with HCC, and it has been reported that the sensitivity and specificity for serum *hTERT mRNA* in detecting HCC were 77.14% and 100%, respectively, which are higher than the sensitivity and specificity for AFP in the early detection of HCC[171]. In another report, the sensitivity/specificity for serum *hTERT mRNA* in diagnosing HCC was found to be 90.2%/85.4%, which is superior to using alpha-fetoprotein (*AFP*), *AFP-L3*, and des-gamma-carboxy prothrombin (*DCP*) in the diagnosis of HCC at an early stage[172]. Therefore, measuring serum *hTERT mRNA* levels might serve as a potential diagnostic tool for HCC.

Taken together, these findings suggest that *TERT* promoter mutations are among the earliest genetic alterations in hepatocarcinogenesis, occurring at preneoplastic stages and behaving as a "gatekeeper" during the malignant transformation sequence[173,174].

Considering that *TERT* promoter mutations are among the earliest recurrent genetic events in tumorigenesis and are also the most frequent somatic genetic alterations in HCC, telomerase inhibition shows potential as an ideal therapeutic target in treating HCC. Currently, different strategies for telomerase inhibition, such as the use of nucleoside analogs, oligonucleotides, small molecule inhibitors, G-quadruplex stabilizers, immunotherapy, and gene therapy in different cancers, are currently in development, preclinical studies or clinical trials[175].

ARID1A and ARID2

Increasing evidence has demonstrated that the misregulation of ATP-dependent chromatin remodeling complexes (chromatin remodelers) contributes to tumorigenesis[176], tumor heterogeneity[177], and the cellular response to anticancer drugs[178-182]. Among the different ATP-dependent chromatin remodelers, genes encoding SWitch/sucrose nonfermentable (SWI/SNF) complex subunits are now recognized as among the most commonly mutated targets affecting chromatin remodeling, as they are present in 20% of human cancers[183-185]. SWI/SNF chromatin remodeling has been linked to a variety of epigenetic processes,

including roles in maintaining nucleosome positioning and interacting with other chromatin modifiers[186]. The SWI/SNF complexes can be divided into two broad categories based on the presence of the AT-rich interactive domain containing protein 1A-B (*ARID1A/B*) subunits (BAF complex) or *ARID2* and polybromo 1 (*PBMR1*) subunits (PBAF complex)[187].

Recent exome and WGS studies of HCC have shown that recurrent inactivating mutations in SWI/SNF subunits are involved in the molecular basis of hepatocarcinogenesis[101,102,188-190]. However, the functional role and molecular mechanisms underlying these mutations in the initiation and progression of HCC are not yet completely understood. Genes involved in coding for chromatin-modifying proteins are commonly mutated in HCC. In particular, two inactivating mutations in genes encoding subunits of the SWI/SNF complex, and *ARID1A* and *ARID2* have been identified in approximately 10% of HCC cases[101,106,107,189,191]. Therefore, it is not surprising that chromatin remodeling complex alterations might play important roles in the initiation and progression of HCC. Interestingly, the frequency of *ARID1A* and *ARID2* mutations occurring in HCC varies considerably across HCC cases, depending on the different etiologies of the disease. For example, *ARID1A* mutations are significantly more frequent in HCC related to alcohol intake than in tumors of other etiologies[102], and *ARID2* mutations commonly occur in HCV-associated HCC[188,192]. However, several studies did not observe an association between *ARID1A* and *ARID2* mutations and the etiology of HCC. *ARID1A* was mutated in 13% of HBV-associated cases of HCC[189], and *ARID2* mutations were not significantly associated with HCV infections[102]. A recent study also demonstrated that *ARID1A* alterations were not correlated with HBV infection, HCV infection or the heavy use of alcohol[193]. These findings suggest that *ARID1A* and *ARID2* mutations are universally present in association with HCC related to hepatitis virus infection and alcohol intake.

The mechanisms by which mutations in SWI/SNF subunits drive tumorigenesis are unclear. Most *ARID1A* and *ARID2* mutations detected in cancer cells to date are inactivating mutations, suggesting that both proteins function as tumor suppressors[194]. Several possible mechanisms for this effect have been suggested. *ARID1A* has been indicated in preventing DNA entanglements during mitosis. Hence, its mutational inactivation could lead to genomic instability and alter gene expression, which could contribute to tumorigenesis[195]. In addition, it has been found that *ARID1A* mutations tend to interact with the activation of the PI3K/AKT pathway in promoting tumorigenesis in many human cancers of diverse origins[196-203]. Furthermore, a recent study found that *ARID1A* mutations alone did not cause the development or progression of cancer but that a combination of *ARID1A* inactivation and a PI3K/AKT pathway aberration was sufficient to initiate tumorigenesis[204]. Theoretically, the two mechanisms mentioned above in other solid tumors might also apply to HCC. The functional significance of *ARID1A* and *ARID2* mutations remains to be elucidated in relation to the initiation and progression of HCC.

In a recent study, HCC cases with altered *ARID1A* expression showed inverse correlations with the nuclear localization of P53 and beta-catenin, suggesting that the *ARID1A* pathway might represent an alternative pathway to the *p53* and beta-catenin pathways in HCC. Thus, *ARID1A* might constitute a promising therapeutic target for treating a subset of HCCs[193].

NFE2L2/NRF2 and KEAP1

Oxidative stress involves elevated intracellular levels of reactive oxygen species (ROS) that cause damage to lipids, proteins and DNA[205]. Recent studies have shown that persistent oxidative stress due to elevated ROS levels is associated with carcinogenesis and the progression of cancer[206-210]. The NRF2-KEAP1 pathway is the major regulator of cytoprotective responses to endogenous and exogenous stresses caused by ROS and electrophiles[211,212]. The key proteins within the NRF2-KEAP1 pathway are the transcription factor *NRF2*, which mediates oxidative stress responses, and *KEAP1*, which is a negative regulator of *NRF2* activity.

NRF2 has been traditionally considered a tumor suppressor because of its cytoprotective functions[213]. In fact, accumulating evidence from genetic analyses of human tumors suggests that the deregulation of *NRF2* is a critical determinant in oncogenesis, and somatic mutations of either *NRF2* or *KEAP1* have frequently been detected in a variety of cancer types[107,214-216]. These findings indicate that mutations in *NRF2* and *KEAP1* frequently play important roles in carcinogenesis.

Recent exome sequencing of HCC samples has revealed that the oxidative stress pathway is activated in 12% of HCC patients, primarily as a result of mutations of *NRF2* or *KEAP1*[106]. Numerous genomic studies on cancer have reported somatic mutations of *NRF2* and inactivating mutations of *KEAP1*(6%-10% and 3%-8% of HCC patients, respectively)[102,106,107,138,217,218]. A recent functional experiment found that *NRF2/KEAP1* mutations were present in 71% of early preneoplastic lesions and in 78.6% and 59.3% of early and advanced HCCs[219], respectively, suggesting that the onset of NRF2/KEAP1 mutations is a very early event in rat hepatocarcinogenesis. In contrast, mutations of *NRF2* and *KEAP1* in humans were observed only in advanced HCC and not in premalignant nodules or early HCC, suggesting that these mutations are late events in hepatocarcinogenesis in humans[105,106]. Despite some differences in the role of mutations of *NRF2*

and *KEAP1* between rats and humans, it is evident that the dysregulation of the NRF2/KEAP1 pathway and mutations of these genes play important roles in hepatocarcinogenesis in both species. The NRF2/KEAP1 pathway might contribute to hepatocarcinogenesis through the following mechanisms. First, the NRF2/KEAP1 pathway might cause epigenetic instability, leading to HCC[220]. Second, either *NRF2* acts by itself as a proto-oncogene or *NRF2* or *KEAP1* mutations support the accumulation of additional mutations of proto-oncogenes[215,221]. Third, the NRF2/KEAP1 pathway could alter the chromatin status, leading to abnormal methylation of TSGs, which might contribute to hepatocarcinogenesis[222]. Interestingly, recent analyses of somatic mutations in HCC have revealed that mutations in *NRF2* or *KEAP1* are significantly correlated with the deregulation of the Wnt/β-catenin pathway *via CTNNB1* or *AXIN1* mutations[102,217]. These results suggest that the NRF2/KEAP1 pathway might interact with Wnt/β-catenin signaling to promote hepatocarcinogenesis. Nevertheless, the exact molecular mechanism underlying the role of *NRF2* in the pathogenesis of HCC must still be investigated.

The finding of recurrent mutations in HCC revealed that *NRF2* activation was a driver event in the progression of tumors[102,138]. Collectively, *NRF2/KEAP1* mutations might be involved in the pathogenesis and progression of HCC. The genetic or pharmacologic inhibition of *NRF2* expression/activity in HCC cells increased the anticancer activity of erastin and sorafenib *in vitro* and in tumor xenograft models[223]. Intriguingly, the accumulation of phosphorylated P62, a selective autophagy substrate, was found to cause the persistent activation of *NRF2*, contributing to the development of HCC[223-225]. In addition, in Japanese HCC patients, *NRF2* activation was associated with the phosphorylation of P62 but not with the *KEAP1* status[226].These results suggest that there might be crosstalk between the NRF2/KEAP1 pathway and P62-mediated selective autophagy, and selective *NRF2* inhibitors or inhibitors of the interaction between phosphorylated P62 and *KEAP1* should be developed as potential therapeutic agents against human HCC.

Janus kinase 1

The JAK/STAT signaling pathways have been identified as promoters of carcinogenesis in a subset of HCCs *via* cytokine-induced JAK/STAT pathway activation[28,227,228]. A previous study using single-strand conformational polymorphisms (SSCPs) and direct sequencing reported a low frequency (1/84, 1.2%) of Janus kinase 1 (*JAK1*) mutations in HCC[229]. Recently, a comprehensive whole genome analysis revealed that *JAK1* mutations appeared in 9.1% of HCCs, and the JAK/STAT pathway was altered in 45.5% of HCCs[28]. These findings indicate that the JAK/STAT pathway might act as one of the major oncogenic drivers in HCC and suggest the possibility of its use as a promising therapeutic approach for HCC treatment.

Ribosomal protein S6 kinase polypeptide 3

Ribosomal protein S6 kinase polypeptide 3 (*RPS6KA3*) encodes a component of the RAS/MAPK signaling pathway, *i.e.*, a gene located on chromosome X that encodes ribosomal S6 protein kinase 2 (RSK2). Recurrent mutations in *RPS6KA3* have been found in 2%-9% of HCCs[102,106,189], suggesting that *RPS6KA3* could act as a newly identified potential driver of the pathogenesis of HCC. Specifically, *RPS6KA3* tended to be mutated in poorly differentiated HCCs[230] and was found in HCCs that developed without cirrhosis[102]. In addition, *RPS6KA3* mutations were frequently associated with *AXIN1* mutations[102], suggesting that *RPS6KA3* inactivation might cooperate with Wnt/β-catenin signaling to promote hepatocarcinogenesis.

SIGNALING PATHWAYS IMPLICATED IN HCC

The recurrent mutated genes reviewed above were found to be highly enriched in multiple key driver signaling processes, including telomere maintenance, *TP53*, cell cycle regulation, the Wnt/β-catenin pathway (*CTNNB1* and *AXIN1*), chromatin remodeling (*ARID1A* and *ARID2*), the phosphatidylinositol-3 kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, and oxidative/endoplasmic reticulum stress (*NFE2L2* and *KEAP1*). In the following section, we briefly summarize two of the most common molecular cellular pathways, Wnt/β-catenin and PI3K/AKT/mTOR, in human HCC[28,35,107,231]. Other pathways are summarized in the section above.

Wnt/β-catenin signaling pathway

The WNT/β-catenin pathway can be classified into canonical (β-catenin dependent) and noncanonical (β-catenin independent) pathways[232]. In the absence of Wnt proteins, β-catenin is phosphorylated at amino-terminal serine and threonine residues by casein kinase 1 (CK1) and glycogen synthase kinase 3β (GSK-3β)[233]. β-catenin phosphorylation is facilitated by the axis inhibition protein (AXIN) and adenomatous polyposis coli (APC). Wnt signaling is activated upon Wnt-ligand binding to frizzled receptors (FZD), followed by the cytosolic accumulation of β-catenin through the prevention of *GSK-3β*-mediated phosphorylation of the β-catenin Ser/Thr domain[234]. The absence of β-catenin phosphorylation releases it from the degradation complex composed of APC, AXIN, GSK-3β and CK1, resulting in an accumulation of β-catenin in the cytoplasm[234]. Subsequently, cytosolic β-catenin can translocate to the nucleus to initiate the transcription of target genes through interactions with T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors[234]. Hepatocytes with the nuclear translocation

of β-catenin displayed abnormal cellular proliferation and expressed membrane proteins associated with HCC, metastatic behavior, and cancer stem cells[235].

The deregulation of WNT/β-catenin signaling has been found in 40%-70% of HCC patients[236]. Increasing evidence suggests that the Wnt/β-catenin signaling cascade plays a major role in the pathogenesis of HCC[234,237]. Some studies have suggested possible mechanisms for this role. For example, research has found that the occurrence of HCC may be closely related to allelic loss, chromosomal changes and mutations in Wnt/β-catenin signaling pathway genes[238]. In addition, the Wnt/β-catenin signaling pathway contributes to angiogenesis, infiltration and metastasis in HCC by regulating the expression of angiogenic factors, such as matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), vascular endothelial growth factor-A (VEGF-A), vascular endothelial growth factor-C (VEGF-C) and basic fibroblast growth factor (bFGF)[239]. However, the precise molecular mechanism remains uncertain.

Mutations in exon 3 of the *CTNNB1* gene, which encodes β-catenin, constitute a crucial molecular mechanism leading to the aberrant activation of the Wnt/β-catenin pathway, which is strongly associated with hepatocarcinogenesis[240]. In addition to gain-of-function mutations in positive modulators of Wnt signaling, such as β-catenin, the Wnt pathway can be activated by loss-of-function mutations in negative modulators, such as *AXIN* and *APC*[241]. It has been suggested that *AXIN* might play an important role in the pathogenesis and progression of HCC *via* the Wnt signaling pathway[242]. Moreover, the overexpression of the Frizzled-7 (FZD-7) receptor and glycogen synthase kinase-3 (GSK-3) inactivation may also lead to aberrant β-catenin pathway activation[243] as the FZD-7 receptor has been found to be up-regulated in 90% of human HCCs[244,245], suggesting that the consequent activation of Wnt/Frizzled-mediated signaling plays a key role in hepatic carcinogenesis. Specifically, one study analyzed the spectrum of mutations in a series of 125 cases of HCC, and the authors identified significant associations between mutations in *ARID1A, RPSK6KA3* or *NFE2L2* and mutations in *CTNNB1* or *AXIN1*, suggesting that Wnt/β-catenin signaling might interact with oxidative stress responses, chromatin remodeling or the RAS/MAPK pathway to promote hepatocarcinogenesis[217].

Mutations in the Wnt/β-catenin pathway have been described in 20%-40% of HCCs[246]. In HBV-related HCC, *β-catenin* mutations have been found at a lower frequency[103,246,247], whereas higher incidences of *β-catenin* mutations have been shown to occur mainly in alcohol- and HCV-related HCCs[101,102,188,248]. These findings suggest that *β-catenin* mutations are associated with the etiology of the HCC, which might be explained in part by actions of the HCV core protein synergizing Wnt-induced stabilization and the accumulation of β-catenin, perhaps playing an important role in the pathogenesis of HCV[249]. In HCC occurring in association with HBV, patients display β-catenin activation, which is induced in a mutation-dependent manner by the expression of the HBx protein[250]. Furthermore, one explanation for why *β-catenin* mutations tend to occur in non-HBV-associated casesis that *AXIN* mutations (and rarely *β-catenin* mutations) are mainly found in chromosome-unstable tumors associated with HBV infections, and *β-catenin* mutations are mainly found in non-HBV, well-differentiated, chromosome-stable tumors[251]. Thus, these two components of the Wnt pathway, β-catenin and *AXIN1*, could operate in distinct ways in human HCC[252].

The verdict on the role of *β-catenin* mutations in the initiation and progression of HCC is currently uncertain. A few studies have demonstrated that *β-catenin* mutations are found only in association with HCC and not in DNs[104,253,254].These results suggest that *β-catenin* mutations might be a late event in malignant progression rather than *β-catenin* being an early event gene or a gatekeeper gene in the multistep process of hepatocarcinogenesis. Nevertheless, another study concluded that β-catenin accumulates in the cytoplasm and the nuclei in precancerous lesions of the liver and might contribute, at least in part, to hepatic carcinogenesis[255]. Moreover, a clonality analysis predicted that the *CTNNB1* mutation was clonal and occurred earlier during hepatocarcinogenesis[256]. To date, numerous studies have investigated the possible mechanisms underlying the role of *β-catenin* mutations in the initiation and progression of HCC. For example, *CTNNB1* mutations are likely to occur as late events in the context of aflatoxin exposure and chronic HBV infection, whereas *CTNNB1* mutations might represent early events in carcinogenesis without a typical mutational pattern in areas with low AFB1 intake[135]. Transcription complexes, formed by a combination of intranuclear β-catenin and transcription factors, activate downstream target genes and regulate the expression of corresponding genes, leading to HCC tumorigenesis[257]. Although a *β-catenin* mutation might represent an important event leading to tumorigenic changes in hepatocytes, several studies using transgenic animal models have shown that the overexpression of mutant or stable forms of *β-catenin* on its own is not sufficient to induce HCC[258,259]. A recent study found that the up-regulated genes v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog G (*MAFG*) and synovial sarcoma, X breakpoint 1 (*SSX1*) significantly synergized with the transcriptional activity of β-catenin, and the overexpression of the downregulated genes one cut homeobox 1 (*Onecut1*) and forkhead box protein A3 (*FOXA3*) potently inhibited the growth of a *CTNNB1*-mutation-positive (HepG2) cell line and negative (Huh-7 and Hep3B) cell lines[260]. In another study, the over-expression of cysteine-rich protein 61 (Cyr61/CCN1) was positively correlated with increased

levels of β-catenin in human HCC samples, indicating that Cyr61 is a direct target of β-catenin signaling in HCC[261]. Therefore, the findings of these studies indicate that *β-catenin* mutations can interact with other oncogenic alterations or pathways to result in hepatocarcinogenesis more often than previously recognized.

Similarly, there have been conflicting data in the literature on the question of whether *β-catenin* mutations in HCC are associated with favorable or unfavorable prognoses[262]. Some studies have found associations of *β-catenin* mutation or activation with worse outcomes, such as moderately/poorly differentiated HCV-related HCC, larger tumor sizes, multiple nodules and increased vascular invasion[263,264]. In contrast, other studies have reported that HCCs harboring *β-catenin* mutations had better outcomes, such as less invasive and less frequent portal vein involvement[138,260,265-268]. A recent meta-analysis also revealed that *β-catenin* mutations could predict a favorable prognosis in patients with HCC[269]. In addition, one study reported that *β-catenin* mutations were not associated with prognoses in patients with advanced HCC[238]. Interestingly, the expression of the noncanonical *Wnt5a*, which is known to inhibit canonical Wnt signaling, was increased in poorly differentiated HCC cell lines[270]. Based on this result, the authors proposed that canonical and noncanonical Wnt pathways play complementary roles in HCC, with canonical signaling contributing to tumor initiation and noncanonical signaling contributing to tumor progression[270]. Accordingly, the noncanonical activation of *Wnt* in HCC deserves further research. Furthermore, a possible mechanism underlying *β-catenin* mutations with favorable outcomes was proposed in another study. In this study, the presence of cytokeratin 19 (CK19) expression or the absence of *β-catenin* mutations was found to be predictive of early tumor recurrence (ETR), and CK19 expression abolished the suppressive effects of *β-catenin* mutations on the progression of HCC. CK19 expression and *β-catenin* mutations were found to play dramatically opposite roles in vascular invasion, ETR and the prognosis of HCC patients[271].

Considering these findings, future prospective studies to determine the initiation, progression and outcome of HCC as a function of the WNT/β-catenin pathway will be essential. Specifically, such studies should consider the geographical origin, etiology and heterogeneity of the patients as well as the modes of WNT/β-catenin pathway activation[272].

PI3K-AKT-mTOR pathway

The phosphoinositide 3-kinase-AKT-mammalian target of rapamycin (PI3K-AKT-mTOR) pathway is one of the most frequently deregulated pathways in human cancers, and it is a master regulator of processes that contribute to tumorigenesis and tumor maintenance[273]. The membrane lipid phosphatidylinositol 4, 5-bisphosphate (PIP2) is phosphorylated by *PI3K* into phosphatidylinositol 3, 4, 5-triphosphate (PIP3), which binds to and activates the serine/threonine kinase *AKT*[274]. The tumor suppressor gene product *PTEN* deleted on the chromosome is antagonistic to *PI3K* activity; the inactivation of *PTEN* through gene deletion increases PIP_3 levels and activates *AKT*, which inhibits apoptosis, leading to the development of tumors[275]. Activated *AKT* initiates a cascade of downstream signaling events, including the mTOR pathway. Once activated by *AKT, mTOR* promotes cell growth and proliferation by stimulating protein synthesis through the phosphorylation of 4E-BPs and the S6 kinases[275].

The PI3K/AKT/mTOR pathway is frequently deregulated in human hepatocarcinogenesis[276]. Furthermore, the deregulation of key genes of the PI3K/AKT/mTOR pathway has clinical importance in HCC[277,278]. As a negative regulator of the PI3K/AKT/mTOR pathway, *PTEN* is considered a tumor suppressor gene. *PTEN* mutations rarely occur in HCC, whereas *PTEN* heterozygosity, resulting in reduced *PTEN* expression, has been observed in 32%-44% of HCC patients[279]. Recent studies have demonstrated that the underexpression of *PTEN* is associated with poorly differentiated HCC, advanced TNM (tumor-node-metastasis) stage and intrahepatic metastasis, and poor patient survival[278,280-282]. *PI3KCA* is an upstream regulator of *AKT*, although there is some controversy regarding the role of *PI3KCA* mutations in HCC. A recent study identified *PIK3CA* mutations in 14% of patients.These mutations were strongly correlated with tumor size, suggesting that *PIK3CA* mutations could be used as prognostic markers in HCC[283]. However, other more recent studies have shown that hot spot mutations in *PIK3CA* are completely absent or rare in HCC[263,284-287]; *PI3K* mutations were not associated with either hepatic carcinogenesis or the postoperative prognosis of HCC patients[284,285,288].

AKT, also known as protein kinase B, is a central effector in the PI3K pathway. Many HCCs have demonstrated the activation of *AKT*, and it has been reported that both hepatitis B and hepatitis C could activate PI3K/AKT signaling[289]. It is well established that *AKT* plays a key role in tumorigenesis by stimulating cell proliferation and inhibiting apoptosis. The phosphorylation of *AKT* at S473 was detected in up to 71% of HCC samples and was associated with the invasion, metastasis, and vascularization of HCC[278]. As an *AKT* effector, S6 ribosomal protein (pS6) could be used as a prognostic indicator of HCC[290]. In addition, phospho-AKT (*pAKT*) expression showed a significant correlation with decreased OS[291], suggesting a worse prognosis for HCC patients with activated *AKT*[292].

mTOR is a key component of the *PI3K* and *AKT* pathways that activate downstream kinases required for G1 to S phase transition[293]. *mTOR* deregulation

has been reported to play a significant role in the pathogenesis and progression of HCC. A recent study showed that high *mTOR* expression levels were correlated with Edmondson tumor grades and cirrhosis[294]. Additionally, data from preclinical studies have indicated that the deregulated expression of mTOR pathway effectors occurred in 40%-50% of HCCs, and the activation of the mTOR pathway was associated with less differentiated tumors, earlier tumor recurrence, and lower survival rates[290,295]. *mTOR* acts by directly activating p70S6 kinase (p70S6K/S6K1) and inhibiting 4E binding protein 1 (4E-BP1)[296]. *mTOR* forms two multiprotein complexes, called *mTORC1* (*mTOR* complexed with raptor) and *mTORC2* (*mTOR* complexed with rictor)[297]. Both *mTORC1* and *mTORC2* participate in regulating the migration and invasion of HCC cells[298]. A recent study showed that a high ratio of the levels of rictor and raptor mRNAs in tumors was an independent prognostic indicator of DFS[297]. This finding suggests that an analysis of *mTOR* expression in cancer tissues could serve as a predictive marker of HCC recurrence after curative treatment.

Currently, many inhibitors targeting the PI3K/AKT/mTOR pathway are being evaluated for treating HCC in preclinical and clinical studies[299,300]. It is hoped that the efficacy of inhibitors of the PI3K/AKT/mTOR pathway, in combination with other anticancer agents, might represent a promising new strategy for treating HCC patients.

PROBLEMS AND PERSPECTIVES

Although numerous genes are altered in association with HCC, only a small number of them are considered alterations that drive clonal expansion and invasion. Most of the somatic alterations appear to be passengers that are neutral for tumor cell selection[301]. So far, most of the genetic events that initiate HCC remain unknown. Therefore, the identification of key driver genes in HCC is crucial to elucidating the genetic mechanism of hepatocarcinogenesis and providing new molecularly targeted therapies for HCC patients.

Recent advancements in NGS technology have allowed for the identification of recurrently mutated genes in the pathogenesis of HCC. For example, a recent study of NGS analyses was performed to identify mutations in the *TERT* promoter, *TP53*, and *CTNNB1* genes that are major drivers of the development of HCC[103]. To date, however, no potential drivers of specific oncogenes (oncogene addiction, which is a term used when a cancer cell is found to be dependent on a single gene to survive) corresponding to targeted therapies have emerged, likely due to the genomic heterogeneity of HCC. In addition, the most prevalent of the critical driver mutations that have been identified in HCC are not yet drug-accessible targets[302]. Although several molecularly targeted agents have been evaluated in clinical trials in advanced HCC, no novel, fully effective molecularly targeted agents for the treatment of patients with advanced HCC have been produced, except for sorafenib. There are two factors, *i.e*., the lack of a clearly identified driver oncogene and the presence of underlying cirrhosis, that are primarily responsible for the frequently unsuccessful results in studies on the use of novel drugs in treating HCC[303].

It is anticipated that studies including large sample sizes combined with the integration of multiple levels of data, such as data on genomic instability, SNPs, and somatic mutations, in conjunction with integrative functional genomic approaches, will contribute to identifying driver genes in the pathogenesis of HCC. The identification of these driver genes will lead to the development of effective molecularly targeted therapies and personalized medicine.

Currently, it has been widely realized that signaling pathways, rather than individual genes, govern the course of carcinogenesis[304]. In fact, HCC is considered a multigenic disease with a multifactorial etiology, and hepatocarcinogenesis is an extremely complex multistep process, in which multiple signaling pathways are altered to some extent. In brief, due to the high complexity and heterogeneity of HCC genomes, it is important to emphasize that identifying the altered signaling pathways implicated in HCC, rather than individual mutated genes, may be the key in elucidating the genetic mechanisms underlying hepatocarcinogenesis. Furthermore, insights into the key signaling pathways will likely aid in defining previously unrecognized oncogenic addiction loops in HCC and in developing more effective targeted therapies[305]. Recent extensive research has identified multiple signaling pathways implicated in the pathogenesis of HCC; however, unfortunately, no single dominant signaling pathway is specifically altered in HCC. Future investigations into associated signaling pathways should elucidate the crosstalk between different signaling pathways, *i.e*., how different signaling pathways interact and how they are coordinately regulated in HCC. It is hoped that targeting these crosstalk pathways will result in superior clinical efficacy in treating HCC patients.

Taken together, current evidence suggests that there are no major mutated genes and signaling pathways corresponding to the development of tumors in the majority of cases of HCC, which might primarily be due to the heterogeneity in their geographic and etiologic backgrounds. Due to the intertumor and intratumor heterogeneity of HCCs, future studies must evaluate in detail genetic alterations in relation to the geographic origin of the disease, both across and within individual patients, and chronologically during tumor progression[306]. At the same time, the geographic and etiologic backgrounds of cases of HCC should also be considered in the design of future clinical trials testing molecularly targeted therapies. Such consideration will aid in identifying personalized

therapies for treating HCC patients.

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