Aberrant regulation of Wnt signaling in hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies in the world. Several signaling pathways, including the wingless/int-1 (Wnt) signaling pathway, have been shown to be commonly activated in HCC. The Wnt signaling pathway can be triggered via both catenin β1 (CTNNB1)-dependent (also known as “canonical”) and CTNNB1-independent (often referred to as “non-canonical”) pathways. Specifically, the canonical Wnt pathway is one of those most frequently reported in HCC. Aberrant regulation from three complexes (the cell-surface receptor complex, the cytoplasmic destruction complex and the nuclear
CTNNB1/T-cell-specific transcription factor/lymphoid enhancer binding factor transcriptional complex) are all involved in HCC. Although the non-canonical Wnt pathway is rarely observed in HCC, two main non-canonical pathways, Wnt/planar cell polarity pathway and Wnt/Ca²⁺ pathway, participate in the regulation of hepatocarcinogenesis. Interestingly, the canonical Wnt pathway is antagonized by non-canonical Wnt signaling in HCC. Moreover, other signaling cascades have also been demonstrated to regulate the Wnt pathway through crosstalk in HCC pathogenesis. This review provides a perspective on the emerging evidence that the aberrant regulation of Wnt signaling is a critical mechanism for the development of HCC. Furthermore, crosstalk between different signaling pathways might be conducive to the development of novel molecular targets of HCC.

**Key words:** Hepatocellular carcinoma; Wingless/int-1; Catenin β1; Crosstalk; Canonical wingless/int-1 signaling; Non-canonical wingless/int-1 signaling

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**Core tip:** The development of hepatocellular carcinoma (HCC) is regarded as a multistage process in which multiple genetic alterations are necessary. The wingless/int-1 (Wnt) pathway is a signaling mechanism that is frequently activated in HCC, especially the canonical Wnt pathway. Moreover, two main non-canonical pathways are also involved in the regulation of hepatocarcinogenesis. Interestingly, the non-canonical Wnt pathway could antagonize the canonical Wnt pathway in HCC. Crosstalk between other signaling pathways and the Wnt pathway has also been shown to promote tumorigenesis. This review highlights the details regarding the Wnt pathway in HCC, which might provide new potential targets for HCC prevention and therapy.

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**INTRODUCTION**

Hepatocellular carcinoma (HCC), the fifth most common malignancy in men and ninth among women worldwide, is the second leading cause of cancer deaths[1]. There are over half a million new cases diagnosed per year[1]. The pathogenesis of HCC involves a complex multistep process that derives from the accumulation of aberrant genetic and epigenetic changes and the dysregulation of certain signaling pathways[2-4], including the wingless/int-1 (Wnt) signaling pathway.

Wnt signaling plays crucial roles in the regulation of diverse processes, including cell proliferation, survival, migration and polarization, embryonic development, specification of cell fate, and self-renewal in stem cells[5]. Aberrant activation of Wnt signaling may contribute to numerous malignancies, such as colon cancer[6,7], gastric cancer[8], esophageal cancer[9], HCC[10], and others. Approximately 95% of observed HCC cases showed deregulation of the Wnt signaling cascade[11].

The Wnt signaling pathway is activated via both catenin beta 1 (CTNNB1)-dependent (also known as “canonical”) (Figure 1) and CTNNB1-independent (often referred to as “non-canonical”) pathways (Figure 2). It is suggested that abnormal regulation of the canonical Wnt signaling pathway is a major and early carcinogenic event[12]. The role of the non-canonical Wnt signaling pathway in HCC is also uncertain. Some studies have shown that non-canonical Wnt signaling is activated in HCC[11,13]. However, others have demonstrated that non-canonical Wnt ligands antagonized canonical Wnt signaling[14,15] and inhibited HCC cell proliferation and migration[13]. Here, we present the general molecular pathology of both the canonical and the non-canonical Wnt signaling pathways, and also the crosstalk between distinct signaling cascades and the Wnt signaling in HCC. This will provide potential clinical implications in finding effective therapeutic targets.

**WNT SIGNALING PATHWAY**

**Canonical Wnt signaling**

Wnt proteins, which are highly conserved in metazoan, are a family of 19 secreted glycoproteins[16]. The canonical Wnt signaling pathway is operated by stabilizing the transcriptional co-activator CTNNB1 through preventing its phosphorylation-dependent degradation. In a normal steady state, there are two pools for CTNNB1 in cells. One is known to interact with the cell adhesion molecule cadherin 1 (CDH1) at the cell-cell junction. The second is present in the destruction complex in cytoplasm, which is assembled by the scaffold proteins AXIN, the human tumor suppressor adenomatous polyposis coli (APC), glycogen synthase kinase 3 beta (GSK3B, also known as GSK3β), and casein kinase 1 alpha 1 (CSNK1A1)[17].

The second pool assembly maintains the low level of CTNNB1 in cytoplasm through phosphorylation of CTNNB1 at serine-45 (Ser45), Ser33, Ser37 and threonine-41 by CSNK1A1 and GSK3β in the destruction complex[18,19]. Phosphorylated CTNNB1 is subsequently recognized and ubiquitinated by the beta-transducin repeat containing E3 ubiquitin protein ligase (BTRC). BTRC is a component of an E3 ubiquitin ligase. This process results in the proteasomal degradation of the phosphorylated CTNNB1[20]. In the absence of nuclear CTNNB1 translocated from the
cytoplasm, T-cell-specific transcription factor (TCF)/lymphoid enhancer binding factor (LEF) proteins act as transcriptional repressors by binding to Groucho/transducin-like enhancers of split 1 (TLE1) proteins. The proteins interact with histone deacetylases, leading to the transcriptional silencing of chromatin.

In conclusion, three complexes are involved in the dynamic activating event: (1) the cell-surface receptor complex; (2) the destruction complex in the cytoplasm; and (3) the CTNNB1/TCF/LEF transcriptional complex in the nucleus. In a normal steady state, there are two pools for CTNNB1 in cells. One is known to interact with CDH1 at the cell-cell junction. The second is present in the destruction complex in the cytoplasm, which is assembled by the scaffold proteins AXIN, APC, GSK3β, and CSNK1A1. CSNK1A1 and GSK3β phosphorylate CTNNB1 in the AXIN complex. Phosphorylated CTNNB1 is subsequently recognized and ubiquitinated by BTRC. In the absence of nuclear CTNNB1 translocated from the cytoplasm, TCF/LEF proteins bind to DNA and act as transcriptional repressors by binding to TLE1 proteins. These in turn interact with histone deacetylases whose activities lead to the transcriptional silence of chromatin.

The binding of Wnt to FZDs, which form the cell-surface receptor complex, promotes the binding of scaffold proteins such as DVL to the FZD intracellular domains. This subsequently induces the aggregation and phosphorylation of LRP6 and the translocation of AXIN. Phosphorylated LRP6 also recruits AXIN to LRP6 on the plasma membrane. This allows AXIN to be inactivated, which then inhibits CTNNB1 phosphorylation. This in turn allows CTNNB1 to escape degradation, accumulate in the cytoplasm, and translocate to the nucleus.

In the nucleus, CTNNB1 interacts primarily with members of the TCF/LEF family of transcription factors and triggers the activation of multiple intracellular signaling cascades. This results in the regulation of various cellular functions. CTNNB1: Catenin beta 1; TCF/LEF: T-cell-specific transcription factor/lymphoid enhancer binding factor; CDH1: Cell adhesion molecule cadherin 1; APC: Adenomatous polyposis coli; GSK3β: Glycogen synthase kinase 3 beta; CSNK1A1: Casein kinase 1 alpha 1; FZD: Frizzled class receptor; BTRC: Beta-transducin repeat containing E3 ubiquitin protein ligase.

Figure 1. Canonical wingless/int-1 signaling pathway. Three complexes are involved in the dynamic activating event: (1) the cell-surface receptor complex; (2) the destruction complex in the cytoplasm; and (3) the CTNNB1/TCF/LEF transcriptional complex in the nucleus. In a normal steady state, there are two pools for CTNNB1 in cells. One is known to interact with CDH1 at the cell-cell junction. The second is present in the destruction complex in the cytoplasm, which is assembled by the scaffold proteins AXIN, APC, GSK3β, and CSNK1A1. CSNK1A1 and GSK3β phosphorylate CTNNB1 in the AXIN complex. Phosphorylated CTNNB1 is subsequently recognized and ubiquitinated by BTRC. In the absence of nuclear CTNNB1 translocated from the cytoplasm, TCF/LEF proteins bind to DNA and act as transcriptional repressors by binding to TLE1 proteins. These in turn interact with histone deacetylases whose activities lead to the transcriptional silence of chromatin.

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Functionally, the Wnt signaling cascade can be activated through several pathways via stimulation of distinct Wnt receptors. In vertebrates, ten members of the frizzled class receptor (FZD) family of proteins comprise a series of seven-pass transmembrane receptors that have been identified as Wnt receptors. In addition to FZD proteins, single-pass transmembrane proteins, such as the low density lipoprotein receptor-related protein (LRP) 5 and LRP6, have been reported to function as Wnt receptors in the canonical Wnt pathway.

The binding of Wnts to FZDs which form the cell-surface receptor complex promotes the binding of scaffold proteins, such as disheveled (DVL) proteins, to the FZD intracellular domains. This, in turn, induces the aggregation and phosphorylation of LRP6 and the translocation of AXIN.

Phosphorylated LRP6 also recruits AXIN to LRP6 on the plasma membrane. This allows AXIN to be inactivated, which then inhibits CTNNB1 phosphorylation. As a result, CTNNB1 succeeds to escape degradation, accumulate in the cytoplasm, and translocate to the nucleus.

Non-canonical Wnt signaling
Non-canonical Wnt pathways are triggered by seve-
ral possible mechanisms, which are all independent of CTNNB1-TCF/LEF transcriptional function (Figure 2). Among these non-canonical Wnt signaling pathways in vertebrates, the Wnt/planar cell polarity (PCP) pathway and the Wnt/Ca\(^{2+}\) pathway have been described in the most detail to date.

**Wnt/PCP pathway:** This pathway is often initiated by Wnt5a and Wnt11 through FZD and DVL. Next, small GTPases, such as ras-related C3 botulinum toxin substrate 2 (RAC1) and ras homolog family member A (RHOA), are activated by formation of the DVL-RAC1 complex and the DVL-dishevelled associated activator of morphogenesis 1-RHOA complex, respectively. The DVL-RAC1 complex then activates c-Jun N-terminal kinase (JNK). Finally, the triggered RHOA leads to Rho-associated coiled-coil containing protein kinase (Rock) activation. This pathway regulates cell polarity in morphogenetic processes, including gastrulation and neural tube closure\(^{32-34}\).

**Wnt/Ca\(^{2+}\) pathway:** In this pathway, Wnt5a/FZD2 activates phospholipase C via the heterotrimeric G proteins. This leads to the generation of dystroglycan 1 and inositol-trisphosphate 3, which increase the intracellular Ca\(^{2+}\) flux and levels. The Wnt/FZD complex also activates cyclic GMP-specific phosphodiesterase (PDE6), and...
then increases intracellular Ca\textsuperscript{2+} concentration through the depletion of cellular cGMP and the inactivation of cGMP-dependent protein kinase. Ca\textsuperscript{2+} activates calcium/calmodulin-dependent protein kinase II and protein kinase C, which in turn inhibits the canonical Wnt pathway. This leads to a wide variety of effects, such as tissue separation during gastrulation in vertebrates and ventral patterning in Xenopus species, as well as cell adhesion, migration, neurodegeneration, inflammation, and tumorigenesis\textsuperscript{35-37}.

Wnt/Receptor tyrosine kinases pathway: The receptor tyrosine kinases (RTK) of the receptor-like tyrosine kinase (RYK) and RAR related orphan receptor A (RORA/ROR2) families function as extracellular Wnt-binding domains and are implicated in Wnt signaling\textsuperscript{38}.

Wnt/Ryk pathway: Ryk binds to Wnt-induced repulsion of axons and mediates cell migration in Drosophila and mice. SRC kinase may act downstream of Ryk in flies, wherein it was originally identified as Derailed\textsuperscript{39}.

Wnt/Ror2 pathway: Wnt5a/ROR2 activates the phosphatidylinositol 3-kinase (PI3K)-cell division cycle 42 (CDC42)-mitogen-activated protein kinase kinase 1 (MEK1)/mitogen-activated protein kinase (MAPK) pathway, resulting in the activation of activating transcription factor 2 and c-Jun and the expression of PAPC\textsuperscript{39}. ROR2 also binds to the actin-binding protein filamin A and promotes filopodia formation\textsuperscript{40,41}. The Wnt5a/ROR2 pathway inhibits the canonical Wnt pathway\textsuperscript{36}.

Wnt/casein kinase I epsilon/TERF2 interacting protein (Rap1) pathway: Wnt8 activates casein kinase I epsilon (CSNK1E), which enhances the phosphorylation and degradation of signal-induced proliferation-associated 1 like 1 (SIPA1L1), a Rap1-specific GTPase-activating protein. Rap1 is thereby activated in a CTNNB1-independent manner. Rap1 regulates actin cytoskeleton and/or cell adhesion during vertebrate gastrulation\textsuperscript{42}.

Wnt/cyclic adenosine monophosphate/protein kinase A pathway: Wnt1/Wnt7a activates the G protein and adenyl cyclase (AC) to increase cyclic adenosine monophosphate (cAMP) levels, which in turn activates protein kinase A (PKA) and the transcription factor cAMP responsive element binding protein 1 (CREB) and myogenic gene expression\textsuperscript{36}. Wnt3a can also trigger the cAMP/PKA pathway\textsuperscript{43}, which could suppress osteoclast differentiation by PKA-mediated phosphorylation and inactivate the nuclear factor of activated T-cells 1 (NFATC1)\textsuperscript{44}.

Wnt/Dvl/atypical protein kinase C pathway: Wnt/Fzd signaling induces atypical protein kinase C (aPKC) stabilization and activation via interaction with DVL. This pathway can promote axon differentiation mediated by the pulmonary adenoma resistance (PAR) 3/PAR6/aPKC complex\textsuperscript{45}.

Wnt/Gsk3beta/microtubule pathway: Wnt/Dvl increases microtubule (MT) stability through the concomitant inhibition of GSK3beta and activation of JNK. This pathway is involved in the modulation of cytoskeleton dynamics\textsuperscript{46}.

Wnt/mechanistic target of rapamycin pathway: Wnt activates mechanistic target of rapamycin (mtor)-mediated translational regulation in tumorigenesis via inhibiting Gsk3-dependent phosphorylation of tuberous sclerosis 2 (TSC2). DVL, AXIN and APC are all involved in it. Activation of the Wnt/mtor pathway promotes cell growth and tumorigenesis\textsuperscript{47}.

Wnt/Fyn (Fyn proto-oncogene, Src family tyrosine kinase)/signal transducer and activator of transcription 3 pathway: Wnt5/Fzd2 can be triggered by Fyn through its SH2 domain. The activated complex subsequently recruits and phosphorylates signal transducer and activator of transcription 3 (STAT3) on Tyr705 and finally contributes to the epithelial-mesenchymal transition (EMT) program, cellular migration, and tumor metastasis\textsuperscript{48}.

The non-canonical Wnt pathways have also been shown to play critical roles, such as in axon differentiation, cell adhesion, cell proliferation, migration and tumorigenesis, in multi-cellular animals.

**GENETIC MECHANISMS OF WNT SIGNALING IN HCC**

Increasing evidences have shown that the Wnt signaling pathway plays a vital role in HCC\textsuperscript{49-51}, especially the canonical Wnt pathway\textsuperscript{52}. Additionally, two of the main non-canonical pathways (the Wnt/pcp pathway and the Wnt/Ca\textsuperscript{2+} pathway) are also involved in the development of HCC\textsuperscript{52,53}. Interestingly, the canonical pathway is antagonized by non-canonical Wnt signaling in HCC\textsuperscript{14,15}. Moreover, other signaling cascades have also been found to regulate the Wnt pathway through crosstalk\textsuperscript{54-57}.

**Canonical Wnt signal in HCC**

Twenty percent to 90% of HCC cases exhibit CTNNB1 activation\textsuperscript{58}, which promotes cell growth and invasive capability in c-Myc/transformation growth factor alpha transgenic mice\textsuperscript{59}. Simultaneous mutation of CTNNB1 and HRAS leads to 100% incidence of HCC in mice\textsuperscript{60}. However, the molecular mechanism of this process is less clear. As described above, three complexes are involved in the dynamic activation of the canonical Wnt signaling pathway. We discuss this below and
according to the regulation of the complexes, including the cell-surface receptor complex, the cytoplasmic destruction complex, and the nuclear CTNNB1/TCF/LEF transcriptional complex.

**Dysregulation of the cell-surface receptor complex in HCC:** Most of the Wnt ligands and their receptors have been reported to be highly expressed in HCC cell lines. Wnt3, Wnt9a and Wnt10b have displayed strong expression in most HCC cell lines, independent of differentiation status. Wnt2b, Wnt4, Wnt5a, Wnt5b and Wnt7b have been reported as overexpressed in poorly differentiated cell lines, while Wnt8b and Wnt9b have been reported as selectively overexpressed in well differentiated cell lines[64]. Almost all FZD receptors (except FZD9 and FZD10) and two co-receptors have also been reported as overexpressed in HCC cell lines[64]. Furthermore, LRP6 has also been found to be overexpressed in 38% of HCC[61].

It has been reported that HCV core protein correlates with increased Wnt1 and Wnt3a expression in HCC cell lines[62,63]. Interaction between Wnt3a and FZD7 could activate canonical Wnt signaling in different groups of HCC studies[64,65]. FZD7 overexpression has been shown to occur in early HCC and to contribute to enhanced tumor cell migration[65]. Overexpression of LR6P has been shown to lead to hyperactivation of the canonical Wnt signaling pathway and to result in enhanced cell proliferation, cell migration, and invasion of human HCC[66,67].

Altered expressions of several secreted extracellular antagonists of Wnt ligands, such as secreted Frizzled-related proteins (SFRP), Wnt inhibitory factor-1 (WIF-1) and Dickkopf-related protein 3 (DKK-3), have been detected in HCC. Different SFRPs have been reported to bind with Wnt and thereby down-regulate their ability to activate FZD[66]. Numerous studies have shown that hypermethylation induces down-regulation of SFRPs (SFRP1 and SFRP5) and the subsequent activation of canonical Wnt signaling in HCC[68,71]. Down-regulation of WIF-1 and DKK-3 mediated by promoter methylation has also been reported to be a common event in HCC[72,73].

In addition, the scaffold protein DVL, which binds to the FZD intracellular domain to activate canonical Wnt signaling, has been shown to be up-regulated in a c-Myc/E2F transcription factor 1 transgenic mouse model of HCC[74]. The antagonisms of DVL, which negatively regulate the canonical Wnt signaling, including, dishevelled binding antagonist of beta catenin 2 (DACT2)[75], Prickle-1[76] and the human homologue of Dapper 1 (HDPR1)[77], are down-regulated in HCC.

**Abrogation of the cytoplasmic destruction complex and CTNNB1 activation in HCC:** Tumor formation is accelerated in HCC cells with active CTNNB1[78,79]. Nuclear accumulation of CTNNB1 is associated with proliferation in HCC cells, whereas CTNNB1 knockdown reduces migration and invasion of HCC cells[80]. However, the molecular mechanism for CTNNB1 activation in HCC still needs further investigation.

Researchers have reported that different degrees of mutations in CTNNB1 lead to the activation of CTNNB1. Reported mutations in exon 3 of CTNNB1 ranged from 2.8% to 44% in HCC cases[59,81-84]. The most frequently mutated site is Ser45, the principal site for phosphorylation mediated by CSNK1A1[85].

Since abnormal CTNNB1 redistribution has been reported in up to 90% of HCC cases[86], and the mutation rate of CTNNB1 in HCC is unmatched (2.8%-44%), it is implied that other mechanisms in addition to the CTNNB1 mutation are involved in the aberrant regulation of Wnt signaling in HCC. Mutations of the destruction complex members in HCC are also reported to contribute to hepatocarcinogenesis. AXIN1[52,84,86] and AXIN 2[86,87] mutations are observed in 5% to 54.2% and around 2.7%-37.5% of HCC cases, respectively. Conditional disruption of AXIN1 leads to the development of liver tumors in mice[88]. However, inactivating mutations of APC and GSK3β are quite rare in human HCC cases[89]. Nevertheless, deletion of APC showed significant connections to HCC through the activation of CTNNB1[89,90], while overexpression of wild-type APC in HCC cell lines reduces canonical Wnt signaling and results in growth suppression[91]. Elevated levels of inactive GSK3β are also observed in both human HCC tissues and mouse models of HCC harboring CTNNB1 accumulation[92]. Suppression of GSK3β activation by phosphorylation of Ser9 decreases CTNNB1 activity[92].

Actually, wild-type and mutated CTNNB1 transgenic mouse models indicate that abnormal CTNNB1 is not sufficient for carcinogenic transformation[95,96]. More factors are found in hepatocarcinogenesis mediated by Wnt signaling. Increasing evidences show that several etiologic factors which induce HCC might be involved in the aberrant regulation of canonical Wnt signaling, including HBV, HCV, and carcinogen exposure.

**HBV-related HCC:** A previous study has determined that mutations in AXIN1 were correlated with HBV-related HCC, whereas mutations in CTNNB1 were correlated with non HBV-related tumors[97]. This implies that mechanisms other than the mutation of CTNNB1 are involved in HBV-related HCC. However, a recent study has shown that genetic polymorphisms in CTNNB1 might affect tumor development and survival in HBV-related HCC[98]. The HBV x gene (HBx) up-regulates von Willebrand factor C and EGF domains (VWCE/URG11) and binds to APC to displace CTNNB1 from the destruction complex, which in turn activates CTNNB1[99]. Thereby, the canonical Wnt signaling is triggered[100].

**HCV-related HCC:** Inconsistent with the mechanism in HBV-related HCC, CTNNB1 mutation is shown to be
approximately twice as significant in HCV-related HCC compared with other causes\textsuperscript{[101]} . Additionally, more studies have proven the tumor-associated role of Wnt signaling in HCV-related HCC\textsuperscript{[102]} . It has been reported that HCV up-regulates microRNA-155 (miR-155), which promotes the nuclear accumulation of CTNNB1 and an accompanying increase in downstream targets\textsuperscript{[103]} . NSSA protein and core protein of HCV may increase CTNNB1 by activating PI3K and increasing the phosphorylation of GSK3β at Ser\textsuperscript{9} \textsuperscript{83,104,105}.

**Carcinogen exposure-induced HCC:** The increased accumulation of CTNNB1 has been shown in around 45% of aflatoxin B1 (AFB1)-associated HCC cases\textsuperscript{[106]} . Further studies indicate that AFB1 exposure might activate the canonical Wnt signaling pathway by down-regulating miR-34\textsuperscript{[107]} . However, there is also research showing a totally distinct role of AFB1 on CTNNB1. The results suggest that AFB1 down-regulates CTNNB1 in HCC\textsuperscript{[108]} . Moreover, HCC is induced in transgenic mice whose liver tumors showed conditional expression of CTNNB1 at 6 mo after diethyl nitrosamine (DEN) exposure. However, no tumor is formed in wild-type mice at 6 mo after DEN exposure, indicating that overexpression of CTNNB1 accelerates tumorigenesis and progression to HCC following DEN exposure\textsuperscript{[109]}.

**Activation of the nuclear CTNNB1/TCF /LEF transcripational complex in HCC**

The human TCF/LEF family consists of four members: TCF-1, LEF-1, TCF-3, and TCF-4\textsuperscript{[10]} . Increased LEF-1 in HCC tissues is associated with cyclin D1 overexpression in the nuclear compartment\textsuperscript{[110]} .

In our previous review, the role of aberrantly spliced TCF-4 variants in HCC was discussed\textsuperscript{[111]} . Overexpression of TCF-4 in HCC cells up-regulates the expression level of hypoxia-inducible factor-alpha (HIF-2α) under hypoxia\textsuperscript{[112]} . HIF-2α is capable of modulating TCF-4-mediated transcriptional activity by interacting with CTNNB1\textsuperscript{[113]} and of up-regulating the expression of epidermal growth factor receptor (EGFR)\textsuperscript{[112]} . HIF family proteins are involved in the development of HCC via promotion of angiogenesis\textsuperscript{[114]} . EGFR promotes HCC cell proliferation and resistance to anti-cancer drugs\textsuperscript{[115]} . In addition, a dominant-negative form of TCF-4 decreases the expression of c-Myc and cyclin D1 and suppresses the growth of BEL7402 cells\textsuperscript{[116]} . Thirty-three percent of human HCC cases in which shorter survival periods are observed show c-Myc amplification\textsuperscript{[117]} . Both N terminus of HCV NS5A and core protein increase TCF-4-dependent transcriptional activity and subsequently up-regulate the downstream targets, such as c-Myc and cyclin D1 in HCC\textsuperscript{[83,105]}.

**Non-canonical Wnt signaling in HCC**

Rare studies have demonstrated the role of non-canonical Wnt signaling in HCC. Several non-canonical Wnt signaling pathways have been proven to be involved in the regulation of hepatocarcinogenesis, such as the Wnt/PCP pathway\textsuperscript{[53]} and the Wnt/Ca\textsuperscript{2+} pathway\textsuperscript{[15]} . However, different factors induce distinct cell fates within the same pathways.

Cyclin-dependent kinase 14 (CDK14)\textsuperscript{[118]} , which is overexpressed in HCC tissues and confers cell invasive potential\textsuperscript{[119]} , can regulate cell cycle progression and cell proliferation by specifically interacting with members of cyclin proteins, such as cyclin D3 and cyclin Y\textsuperscript{[120,121]} . Studies have demonstrated that CDK14 up-regulated DVL2 and Naked1 in non-canonical Wnt signaling in HCC by forming a direct complex with cyclin Y\textsuperscript{[12]} . Exogenous overexpression of CDK14 and cyclin Y is also able to activate Rho GTPases (RHOA, RAC1, and CDC42) in HCC. The activated Rho GTPases result in the active formation of actin stress fibers\textsuperscript{[15]} , which lead to the modulation of cell motility\textsuperscript{[122]}.

Activation of non-canonical Wnt pathways, under some conditions, could suppress HCC. For instance, Wnt11 is reported to activate RHOA and Rock. Activated Rock subsequently inhibits RAC1 which contributes to decreased cell migration and motility in HCC\textsuperscript{[15]} .

In addition, the same Wnt ligand could also activate different non-canonical Wnt pathways in HCC. Exogenous overexpression of Wnt11 in HCC cells could also increase cytosolic free Ca\textsuperscript{2+}, and subsequently activate PKC, which translocates from the cytoplasm to the plasma membrane\textsuperscript{[15]} .

**Regulation of Wnt signaling by crosstalk in HCC**

**Non-canonical Wnt pathway antagonizes the canonical pathway:** It has been reported that the non-canonical Wnt pathway can inhibit canonical Wnt signaling in other cancers\textsuperscript{[123,124]} . However, this phenomenon is rarely reported in HCC. Non-canonical Wnt ligand Wnt5a has been reported to inhibit TCF activation mediated by activated CTNNB1 in HCC cells\textsuperscript{[12]} . Wnt11, which has been shown to inhibit HCC cell proliferation, antagonizes canonical Wnt signaling through phosphorylation of CTNNB1 and reduction of TCF-mediated transcriptional activity induced by activated PKC\textsuperscript{[15]} .

**Other signaling pathways activate the Wnt signaling pathway:** Accumulating evidences have demonstrated that activation of Wnt signaling can act in concert with other oncopogenes, such as transforming growth factor beta (TGF-β)\textsuperscript{[125]} , hepatocyte growth factor (HGF)/c-Met pathway\textsuperscript{[55]} , HIF-1α/EMT pathway\textsuperscript{[128]} and insulin/insulin-like growth factor-1 (IGF-1) pathway\textsuperscript{[57]} , to promote tumor progression (Figure 3).

Wnt pathway activation may be mediated by TGF-β\textsuperscript{[54,126,127]} . Interactions between the TGF-β and CTNNB1 pathways are crucial for expression of CTNNB1 target genes in HCC\textsuperscript{[126]} . The TGF-β effector Smad3 can promote the nuclear translocation of CTNNB1\textsuperscript{[128]} . Recently, AXIN2 was reported to be
up-regulated by TGF-β treatment in HCC cell lines, resulting in the activation of Wnt signaling\(^\text{[129]}\). βII-spectrin (SPTBN1), an adapter protein for Smad3/Smad4 complex formation during TGF-β signal transduction, is down-regulated in HCC cells\(^\text{[130]}\). Loss of SPTBN1 promotes tumor formation and invasion of HCC cells through suppressing Wnt inhibitor Kallistatin and subsequently promoting CTNNB1 dephosphorylation and nuclear localization\(^\text{[130]}\).

Crosstalk between the HGF/c-Met pathway and the Wnt pathway might also contribute to the progression of HCC. C-Met, a tyrosine kinase receptor of HGF, which can be associated with CTNNB1 at the inner surface of the hepatocyte membrane\(^\text{[131]}\), is often co-activated with CTNNB1 in HCC\(^\text{[132]}\). Co-delivery of c-Met and constitutively active CTNNB1 into mouse livers rapidly induced primary hepatic tumors\(^\text{[132-134]}\). Monga et al\(^\text{[131]}\) have shown that HGF treatment could induce the dissociation of CTNNB1 from c-Met and its subsequent translocation to the nucleus via tyrosine phosphorylation. Further studies have determined that CTNNB1 enhanced c-Met-stimulated focal adhesion kinase (FAK) activation and synergistically induced the activation of the AKT/extracellular receptor kinase (ERK)-Cyclin D1 signaling pathway in a FAK kinase-dependent manner\(^\text{[135]}\). FAK is also reported to be overexpressed in HCC\(^\text{[135]}\) and required for CTNNB1-induced Cyclin D1 expression in a kinase-independent way\(^\text{[135]}\).

EMT is a process of phenotype shifting of cells associated with embryogenesis, inflammation, and cancer metastasis\(^\text{[136]}\). HIF-1α is reported to mediate the hypoxia-induced EMT via up-regulation of transcription effectors such as TCF-3, which suppress CDH1 expression\(^\text{[137]}\). HIF-1α can compete with TCF-4 to bind with CTNNB1 and form the HIF-1α/CTNNB1 complex. Increased HIF-1α activity, in turn, leads to decreased canonical Wnt signaling activity, and consequently enhanced hypoxia-induced EMT in HCC\(^\text{[56]}\).

Studies have demonstrated that the presence of insulin/IGF-1 could result in CTNNB1 stabilization through inhibition of GSK3β activity, which stimulates TCF/LEF-dependent transcription activation\(^\text{[57]}\). The activation of PI3K/Akt and Ras might mediate the inactivation of GSK3β\(^\text{[57]}\).

**CONCLUSION**

Development of HCC is a multistage process precipitated by multiple specific molecular alterations. Several signaling pathways take part in this process, such as the PI3K/Akt pathway, the Wnt pathway, the TGF-β pathway, the HGF/c-Met pathway, and the IGF pathway. Among these, aberrant regulation of the Wnt signaling pathway appears to be an important event leading to inappropriate transcription of various oncogenic target genes. Most importantly, Wnt signaling might play vital roles in hepatocarcinogenesis through crosstalking with several different signaling cascades (Figure 3). However, the molecular mechanisms of the crosstalk in HCC context still demand further investigation.

Considering that targeting the Wnt signaling pathway might provide potential therapeutics in the treatment of HCC, extra studies are still needed.
Our recent study has shown that urolithin A, one of the intestinal metabolites of ellagic acid, exerts antiproliferative and antioxidant effects in HepG2 cells through the inhibition of canonical Wnt signaling[138]. In addition, several types of antagonists, such as peptides, small synthetic compounds, and blocking antibodies, etc. could suppress tumor formation and metastasis by targeting different factors in the Wnt pathway. Some of them target the interaction between the Wnt ligand and the Fzd receptor; some target the destruction complex; the others could target the CTNNB1/TCF/LEF transcriptional complex[5,139-141]. Actually, some commercial medicines for other diseases have been found to modulate the Wnt signaling pathway. For instance, antipsychotic medications like dopamine D(2) receptor antagonist may treat symptoms of psychosis, at least in part, through modulation of the Wnt signaling pathway[142]. The non-steroidal anti-inflammatory drugs aspirin and indomethacin attenuate the canonical Wnt signaling pathway[143]. The cyclooxygenase-2 inhibitor celecoxib can inhibit CTNNB1-dependent transcription in colorectal cells[144] and suppress polyp formation in familial adenomatous polyposis patients[145]. However, these drugs may function through other signaling cascades either. Furthermore, there is still no inhibitor specific to the Wnt signaling pathway that has progressed to HCC clinical therapy. As a result, a better definition of the role of the Wnt pathway in the cascades network during hepatocarcinogenesis may reveal novel molecular targets which might be used for therapy of HCC.

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