



Review Article



Unraveling the Role of the Wnt Pathway in Hepatocellular Carcinoma: From Molecular Mechanisms to Therapeutic Implications

Zixin Liang^{1#}, Shanshan Li^{2#}, Zhiyu Wang¹, Junting Zhou¹, Ziyue Huang³, Jiehan Li⁴, Haolin Bao¹, Judy Wai Ping Yam^{5*}  and Yi Xu^{1,2,5,6,7,8,9,10*} 

¹Department of Hepatopancreatobiliary Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China; ²School of Pharmacy, Anhui Engineering Technology Research Center of Biochemical Pharmaceutical, Bengbu Medical University, Bengbu, Anhui, China; ³Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC), Beijing, China; ⁴Department of Urology, the Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China; ⁵Department of Pathology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China; ⁶Fujian Provincial Key Laboratory of Tumor Biotherapy, Fuzhou, Fujian, China; ⁷Fujian Provincial Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian, China; ⁸Key Laboratory of Clinical Laboratory Diagnosis and Translational Research of Zhejiang Province, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China; ⁹State Key Laboratory of Targeting Oncology, National Center for International Research of Bio-targeting Theranostics, Guangxi Key Laboratory of Bio-targeting Theranostics, Collaborative Innovation Center for Targeting Tumor Diagnosis and Therapy, Guangxi Medical University, Nanning, Guangxi, China; ¹⁰Shanghai Key Laboratory of Molecular Imaging, Shanghai University of Medicine and Health Sciences, Shanghai, China

Received: October 29, 2024 | Revised: December 13, 2024 | Accepted: December 23, 2024 | Published online: January 14, 2025

Abstract

Hepatocellular carcinoma (HCC) is one of the deadliest malignant tumors in the world, and its incidence and mortality have increased year by year. HCC research has increasingly focused on understanding its pathogenesis and developing treatments. The Wnt signaling pathway, a complex and evolutionarily conserved signal transduction system, has been extensively studied in the genesis and treatment of several malignant tumors. Recent investigations suggest that the pathogenesis of HCC may be significantly influenced by dysregulated Wnt/ β -catenin signaling. This article aimed to examine the pathway that controls Wnt signaling in HCC and its mechanisms. In addition, we highlighted the role of this pathway in HCC etiology and targeted treatment.

Citation of this article: Liang Z, Li S, Wang Z, Zhou J, Huang Z, Li J, et al. Unraveling the Role of the Wnt Pathway in Hepatocellular Carcinoma: From Molecular Mechanisms to Therapeutic Implications. J Clin Transl Hepatol 2025;13(4): 315–326. doi: 10.14218/JCTH.2024.00401.

Keywords: Hepatocellular carcinoma; Wnt signaling; Pathogenesis; Proliferation; Invasion; Metastasis; Therapy.

*Contributed equally to this work.

***Correspondence to:** Judy Wai Ping Yam, Department of Pathology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR 999077, China. ORCID: <https://orcid.org/0000-0002-5637-121X>. Tel: +852-22552681, Fax: +852-22185212, E-mail: judyyam@pathology.hku.hk; Yi Xu, Department of Hepatopancreatobiliary Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang 150086, China. ORCID: <https://orcid.org/0000-0003-2720-0005>. Tel/Fax: +852-94791847, E-mail: xuyihb@pathology.hku.hk.

Introduction

Hepatocellular carcinoma (HCC) has steadily become a malignancy with a very high incidence and fatality rate in China, and even globally.^{1,2} After the pathological diagnosis of patients with liver cancer, it was found that the type with the highest incidence was HCC. Annually, around 700,000 new cases of liver cancer are reported worldwide, of which primary liver cancer accounts for about 300,000–400,000 cases, and ninety percent of these patients have HCC.³ The pathogenesis of HCC has not yet been thoroughly studied, but the main causes of HCC are viral infections, long-term alcohol abuse, exposure to liver-damaging toxins, long-term chronic liver damage, and some immune and metabolic diseases.⁴ Due to its high degree of malignancy, invisibility, and inconspicuous onset symptoms, many patients are diagnosed at a more advanced stage, losing the chance for radical treatment by surgery, and can only adopt conservative treatments.⁵ Still, the indications for surgery are limited and restricted to HCC patients with good liver function, appropriate mass size, and no vascular invasion. Conservative treatments for advanced HCC mainly include chemotherapy, immunotherapy, targeted therapy, radiofrequency ablation, and transarterial chemoembolization.^{6,7} However, conservative treatment is not a radical therapy, so the curative effect is unsatisfactory. These treatments are expensive and have many side effects, which bring inevitable physical and mental trauma to patients.⁸ As research on HCC progresses, the understanding of its pathogenesis has deepened, and new ideas and methods for clinical treatment have been proposed. However, despite this, the incidence and five-year survival rate

of HCC have not been significantly improved.⁹ The Wnt signaling pathway plays a crucial role in processes such as cell proliferation, differentiation, migration, and embryonic development.¹⁰ In recent years, an increasing number of studies have revealed the significance of the Wnt signaling pathway in cancer, particularly in the initiation and progression of malignant tumors such as HCC, colorectal cancer, and breast cancer.^{11,12} Aberrant activation of the Wnt pathway has been shown to be a key factor driving tumor cell proliferation, invasion, and metastasis. Recent genomic and transcriptomic analyses have classified HCC into three molecular subtypes: C1, C2, and C3.¹³ In the C1 subtype, Wnt activation is linked to increased hepatocyte proliferation and anti-apoptotic abilities, often through abnormal regulation of β -catenin stability and nuclear translocation.¹⁴ In the C2 subtype, Wnt signaling enhances invasiveness and metastasis by activating the epithelial-mesenchymal transition (EMT), with β -catenin up-regulated.¹⁵ Although less studied in the C3 subtype, Wnt signaling may modulate immune cell function by regulating the immunosuppressive microenvironment in HCC.¹⁶ Therefore, research on HCC remains urgent, and integrating progress in Wnt signaling pathway research and revealing its potential mechanisms can help inspire new research directions and clinical diagnosis and treatment approaches.

Wnt signaling pathway

The Wnt signaling pathway plays a vital role in maintaining the body's homeostasis and physiological balance.¹⁷ Wnt signaling was first revealed in the 1970s and described as an embryonic developmental mutation-associated gene-phenotype. In experimental animal studies conducted at that time, Wnt signaling was found to be associated with mammary tumor gene activation in mice.^{18,19} The first human tumor linked to Wnt signal transduction progresses from familial adenomatous polyposis to colon cancer.²⁰ As a result, an increasing body of research has demonstrated that the Wnt signaling pathway is primarily responsible for initiating intracellular signaling pathways and facilitating cell-to-cell information transfer, contributing to the development of many cancers.^{21,22} The atypical planar cell polarity (PCP) route, the Wnt/ Ca^{2+} pathway, and the canonical Wnt/ β -catenin pathway are the three main branches of the Wnt signaling pathway.²³ Upon learning that the Wnt signaling pathway contributes to the growth of malignancies, researchers began to explore the pathways through which Wnt signaling is regulated. In conjunction with previous studies on Wnt signaling in different tumors, researchers found that extracellular vesicles (EVs) in tumors contribute to tumor progression and metastasis by regulating Wnt signaling. Tumors where this mechanism has been clearly identified include cervical, breast, and colon cancers, among others.^{24,25} According to current research, the primary method by which EVs modulate Wnt signaling is by altering the Wnt/ β -catenin pathway, the traditional route.²⁶ EVs carry miRNAs that regulate the activity of the Wnt signaling pathway by targeting key genes.²⁷ For example, Wu *et al.* examined the methylation status of miR-137 using 1 TCGA data and 3 GEO datasets, confirming that miR-137 inhibits CRC cell proliferation, migration, invasion, and xenograft tumor growth. miR-137 accelerates the degradation of c-Myc and β -catenin through the inhibition of RNF4, which affects protein stability and the inhibition of the Wnt pathway, highlighting its therapeutic potential in CRC.²⁸ Lin *et al.* enhanced the LNC-POTEM-4/miR-149-5p/Wnt4-regulated signaling axis by inhibiting negative regulators of the Wnt pathway (such as adenomatous polyposis coli (APC) and axis inhibition protein (AXIN) or increasing the stability and nuclear

translocation of β -catenin, promoting tumor cell proliferation and metastasis.²⁹ Additionally, EVs can serve as carriers of Wnt signaling molecules, such as Wnt3a and Wnt5a, which bind to Frizzled receptors on recipient cells, activating the Wnt pathway and regulating target gene expression. This mechanism not only facilitates signaling between cancer cells but may also influence the behavior of immune cells and endothelial cells in the tumor microenvironment (TME).³⁰ The majority of patients with hepatocellular carcinoma have verified mutations encoding Wnt components. One such gene is CTNNB1, which encodes the β -catenin protein. Hepatocellular carcinoma development is thought to be closely associated with abnormal activation of this system.^{31,32} CTNNB1 mutations lead to abnormal β -catenin stability and nuclear translocation, enhancing Wnt/ β -catenin signaling activity, promoting tumor cell proliferation, and inhibiting apoptosis, while also affecting the TME.³³ Overactivation of β -catenin can induce the secretion of immune-suppressive cytokines such as transforming growth factor β (TGF- β) and IL-10, inhibit the activity of effector T cells, and promote the accumulation of Treg and MDSC cells, leading to immune evasion.³⁴ Furthermore, mutations in AXIN and glycogen synthase kinase 3 β (GSK-3 β) can sustain Wnt/ β -catenin signaling, enhancing β -catenin stability and further driving tumor growth, while also altering the immune microenvironment, promoting immune evasion and inflammation.³⁵

Wnt/ β -catenin signaling pathway

The Wnt/ β -catenin signaling pathway is composed of three primary components: proteins associated with the Wnt signaling pathway, complexes that regulate the degradation-related fate of β -catenin proteins, and crucial transcriptional regulators found in the nucleus. Each of these components has unique qualities that either prevent or promote cancer.³⁶ The main functions of β -catenin proteins are to act as mediators of intercellular adhesion between neighboring cells and, in the presence of Wnt receptors, to function as key Wnt signaling components, activating downstream gene transduction and responding to nuclear signals.^{37,38} The most important degradation complexes of the pathway typically consist of a complex containing AXIN, GSK-3 β , casein kinase I, and APC, which are capable of phosphorylating and degrading β -catenin proteins to maintain them at a stable level, preventing downstream genes from being transcribed.^{39,40} The degradation complex is simultaneously prepared for action by the activation of the Wnt signaling pathway.⁴¹ Another important aspect is that the control of Wnt signaling activity requires multiple positive and negative receptors, such as Dickkopf-1.⁴² This mechanism helps maintain the typical Wnt signaling pathway in a hypoactive state. Together, these mechanisms keep β -catenin proteins at consistently low levels and prevent Wnt signaling overexpression. Transmembrane Frizzled (FZD), a glycoprotein present on the surface of cells, is the primary ligand that binds to the Wnt classical pathway. Nineteen isoforms of the human Wnt ligand gene are also known to be involved in Wnt signaling on the cell surface. Furthermore, it has been demonstrated that Wnt can selectively recognize FZD on the cell surface and that the expression of Wnt is significantly higher in tumor tissues than in normal tissues.²² The Wnt signaling pathway becomes pathogenic when there is continuous accumulation of β -catenin. One key event that leads to this occurrence is the inhibition of the axonemal protein complex from phosphorylating β -catenin proteins. Cytoplasmic disheveled (DVL) proteins and the co-receptor low-density lipoprotein receptor-associated proteins 5/6 (LRP5/6) are the main mediators of this activity. Accumulated DVL is phosphorylated

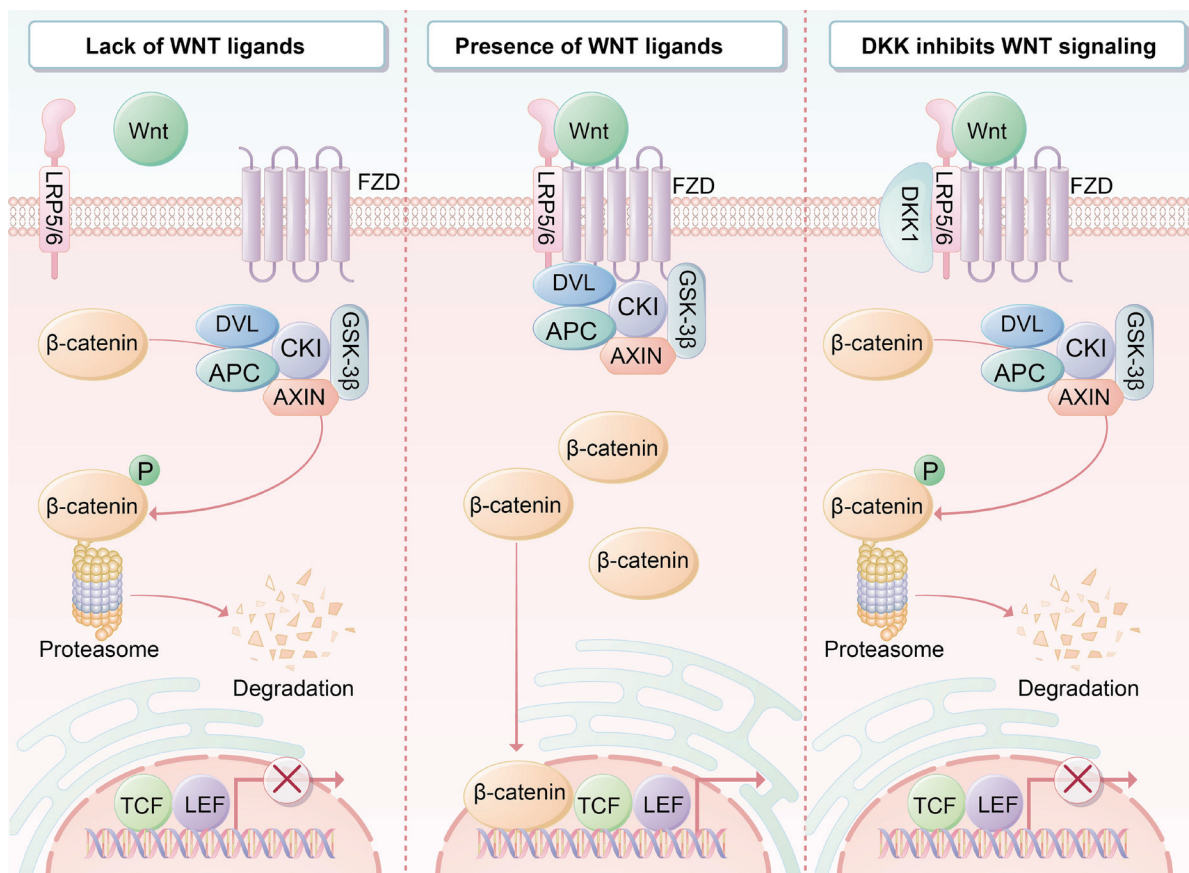


Fig. 1. Wnt/β-Catenin signaling pathway. FZD, frizzled; DVL, dishevelled; APC, adenomatous polyposis coli; CK1, casein kinase 1; AXIN, axis inhibition protein; TCF, T-cell Factor; LEF, lymphoid enhancer binding factor; LRP5/6, low-density lipoprotein receptor-related protein 5/6; DKK1, dickkopf-related protein 1.

in the cytosol by a variety of protein kinases, causing AXIN to lose its degradation function by interacting with DVL and FZD, allowing β-catenin proteins to relocate to the nucleus and carry out a transcriptional function (Fig. 1).^{43–45}

Non-canonical Wnt signaling pathway

Atypical Wnt signaling pathways include the Wnt/PCP and Wnt/Ca²⁺ signaling pathways (Fig. 2). Although researchers have not paid as much attention to these atypical pathways as to the canonical pathways, it has been established that many important physiological and pathological processes in the human body are impacted by abnormal Wnt pathways.⁴⁶ Studies have shown that Wnt3, Wnt5, and Wnt10 are involved in initiating downstream target genes through atypical Wnt signaling pathways.⁴⁷ This finding suggests that atypical Wnt signaling pathways may share similarities with canonical Wnt pathways, meaning anomalies in these pathways and certain route elements may be connected to the initiation and spread of tumors. Additionally, researchers have found that both pathways begin with the Wnt ligand binding to FZD, by comparing their initiation mechanisms. RHO-associated kinase and c-Jun N-terminal kinases are two proteins linked to cytoskeletal control and target gene transcription that are activated when the Wnt/PCP pathway is triggered. This is due to the significant amount of DVL transmission information pulled by the Wnt/PCP pathway.^{48,49} PLC activation increases the breakdown of phosphatidylinositol (4,5)-biphosphates into diacylglycerol and inositol (1,4,5)-triphosphates, which in turn increases cytosolic calcium ion release in response

to Wnt/Ca²⁺ signaling.^{50,51} Cyclic guanosine monophosphate expression is downregulated in response to increased calcium ions, whereas protein kinase C, calcineurin, and calmodulin-dependent protein kinase II are upregulated. Calmodulin-dependent protein kinase II and calcineurin both activate the nuclear factor of activated T-cells, which translocate to the nucleus and is involved in target gene regulation, and Nemo-like kinase, which suppresses the canonical Wnt signaling pathway.^{52–54}

The role of the Wnt signaling pathway in HCC

Involvement in pathogenesis

The Wnt/β-catenin protein signaling pathway plays a significant role in the development of HCC and is essential for regulating several physiological and pathological liver activities. There is approximately a 40% genetic variation between its key components, and aberrant expression and mutations have been demonstrated to be crucial for the development and dedifferentiation of HCC.^{55,56} EVs in HCC maintain a certain level of invasiveness of HCC cells by regulating Wnt signaling and delivering contents between cells, which in turn mediates the biological behavior of the cells and promotes tumor progression.⁵⁷ It has been confirmed that a large amount of abnormally activated Wnt and its contents exist in HCC tissues, further activating downstream target genes through its signaling pathway, which maintains the high invasiveness of HCC.^{58,59}

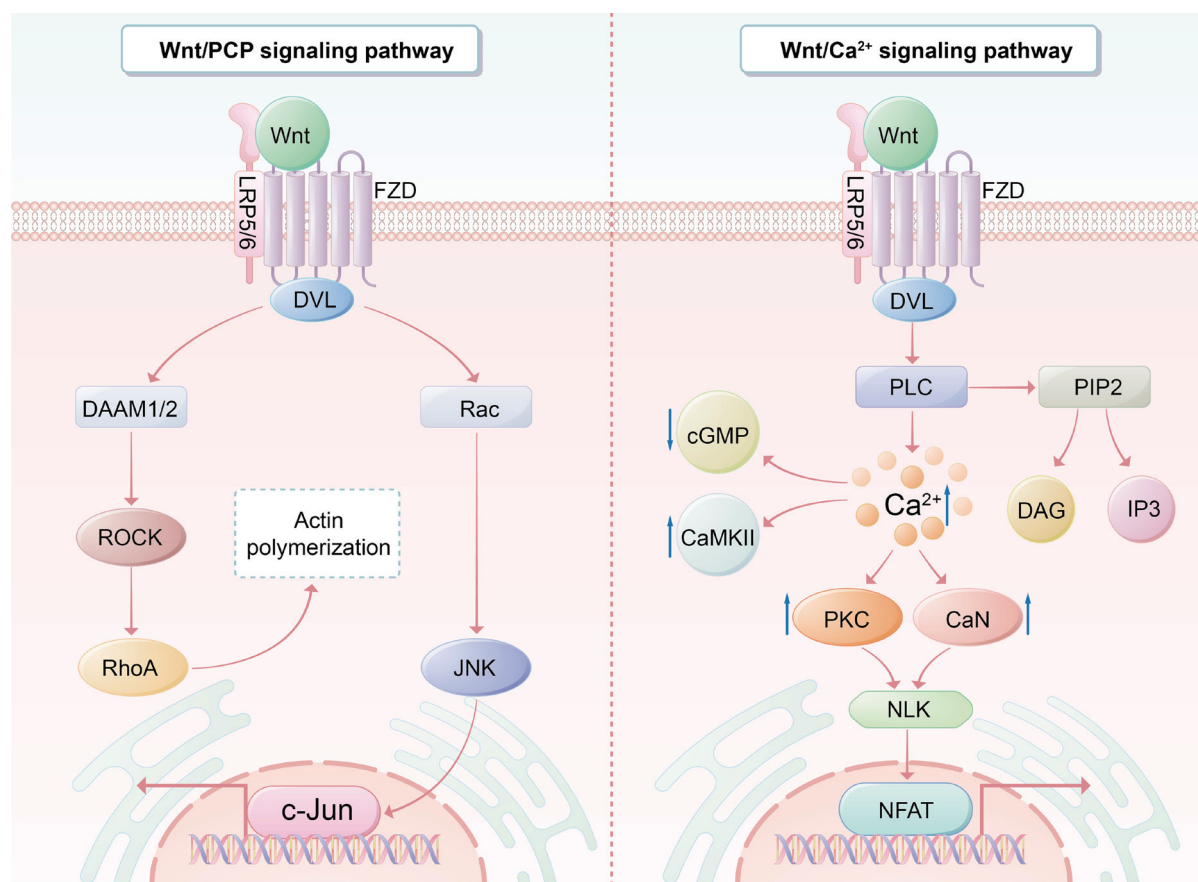


Fig. 2. Non-canonical Wnt signaling pathway. FZD, frizzled; DVL, dishevelled gene; ROCK, Rho-associated coiled-coil kinase; RhoA, Ras homolog gene family member A; DAAM1/2, dishevelled-associated activator of morphogenesis 1/2; JNK, Jun N-terminal kinase C-Jun-N-terminal kinase; PLC, phospholipase C; cGMP, cyclic guanosine monophosphate; DAG, diacylglycerol; IP3, inositol trisphosphate; PKC, protein kinase C; NLK, nemo-like kinase; NFAT, nuclear factor of activated T-cells; ↑, up-regulated expression; ↓, down-regulated expression.

The most common gene mutation in the Wnt/ β -catenin signaling pathway occurs in exon 3 of the β -catenin-encoding CTNNB1 gene.⁶⁰ According to one study, about 30% of the CTNNB1 gene, which codes for β -catenin proteins, is mutated in HCC cells. This mutation interacts with T cell factor (TCF) 4, a transcription factor specific to Wnt cells, to initiate metabolism and proliferation before Wnt response elements are recruited. This results in blocked phosphorylation of β -catenin proteins, which, in combination with mutant histone modifying factors and chromatin remodeling factors, prevents their degradation by the proteasome, allowing the translocation of β -catenin proteins to the nucleus. The findings indicate that a compound containing APC, AXIN1, GSK3 β , and CK1 is responsible for this process.^{61–63} Two of the most well-studied mechanisms of aberrant activation of the Wnt/ β -catenin signaling pathway are cellular mutations and epigenetic modifications. Promoter methylation and the aberrant synthesis of non-coding RNAs are among the other factors. Regional and ethnic factors affect CTNNB1 mutations, with significantly more CTNNB1 mutations found in patients from Asia and Europe compared to HCC patients from Africa.⁶⁴ In addition, etiological factors of HCC also play a role. Research indicates that CTNNB1 mutations are more common in HCC caused by HCV than in HCC caused by HBV, and alcohol-associated HCC has the highest correlation with the frequency of CTNNB1 mutations, approximately 42%.^{65,66} Mutations affecting AXIN, GSK-3 β , and APC, or

their lack of function as AXIN complex constituents, can also trigger the pathway and cause cancer. APC gene inactivation results in cancer through the combined effects of GSK-3 β phosphorylation, hepatic β -catenin overexpression, and AXIN mutations.^{67,68} Zhang *et al.* found in earlier research that the Wnt3/FZD7-mediated β -catenin signaling pathway was more highly expressed in HCC. Subsequent analysis of the effects of Wnt3 binding to the N-glycosylation site-directed mutation of FZD7 on HCC cell development showed that Wnt3/ β -catenin signaling pathway-related protein levels were negatively impacted by downregulating Wnt3's ability to bind to FZD7, which in turn affected cell invasion, migration, and proliferation.⁶⁹ Similarly, related studies have demonstrated that a variety of Wnts act in conjunction with various FZD receptors, overexpressing and decreasing the levels of secreted frizzled-associated proteins in tissues and leading to their methylation in HCC and its surrounding tumor microenvironment. Initially, secreted frizzled-associated proteins functioned to oppose the Wnt/ β -catenin signaling pathway; however, as their quantity decreased and inactivation occurred, the ability of the pathway to be activated increased, gradually accumulating and promoting HCC tumorigenesis.^{70–72} This study has validated two important factors that lead to aberrant activation of the Wnt/ β -catenin protein signaling pathway in HCC tumor formation: the previously reported CTNNB1 gene mutations and the loss of function or mutations in AXIN, GSK-3 β , and APC complexes.^{73,74} Among

these, missense mutations or deletions account for most loss-of-function mutations of GSK-3 β and APC axis protein complexes, resulting in a decrease in the negative regulatory function of the pathway. These also facilitate the translocation of β -catenin proteins from the cytoplasm to the nucleus.^{75,76} Mutations in CTNNB1 are more likely to occur in both ways. Missense mutations or deletions of APC hypermethylate the WNT inhibitory factor-1 promoter, preventing the WNT inhibitory factor-1 from being expressed, causing cells to lose their usual control and encouraging the growth of HCC cells.⁷⁷ Activation of receptor tyrosine kinases, high levels of R-spondins, and TGF- β -dependent activation in HCC are just a few of the ways that the Wnt/ β -catenin signaling pathway is activated. The interaction of multiple HCC-related high-risk factors, either together or separately, leads to HCC tumorigenesis, which is extremely complex. These mechanisms are still under investigation.^{78–80}

Shaping the TIME

The Wnt signaling pathway regulates the immune system in HCC through multiple mechanisms. First, Wnt signaling upregulates immunosuppressive factors such as TGF- β and IL-10, promoting the accumulation of Treg and MDSC cells, which in turn inhibit the function of effector T cells and weaken the anti-tumor immune response.⁸¹ Abnormal activation of the Wnt/ β -catenin pathway can also alter the interactions between HCC tumor cells and immune cells, further facilitating immune escape.⁸² Secondly, Wnt signaling may influence the polarization of tumor-associated macrophages, inducing their conversion to an immunosuppressive phenotype, which enhances immune evasion mechanisms in the TME and impedes the immune system's ability to eliminate the tumor.⁸³ Additionally, the Wnt pathway regulates cytokines and chemokines in the TME, reinforcing the immunosuppressive environment and supporting tumor cell survival and expansion under immune pressure.⁸⁴

Promote malignant biological behavior

Proliferation: When the Wnt signaling pathway is activated, the amount of β -catenin protein increases. This protein can enter the nucleus and bind to TCF or lymphatic factor (LEF) to regulate the production of downstream target genes such as c-Myc and Cyclin D1.^{85,86} These genes are upregulated, which promotes cell cycle progression and boosts cell proliferation.⁸⁷ In HCC cells, the Wnt signaling pathway is often aberrantly activated, releasing β -catenin from the GSK-3 β degradation complex and causing it to accumulate in the cytoplasm.⁸⁸ Subsequently, β -catenin penetrates the nucleus and associates with TCF/LEF family members to form a transcriptional complex.⁸⁹ A cell's Frizzled and LRP5/6 receptors are the sites where Wnt ligands bind to activate the Wnt signaling pathway, which in turn inhibits GSK-3 β activity.⁹⁰ GSK-3 β is typically responsible for phosphorylating β -catenin, which accelerates its breakdown.⁹¹ GSK-3 β inhibition prevents β -catenin from being phosphorylated and eliminated, causing it to accumulate in the cytoplasm.⁹² The accumulating β -catenin is then translocated to the nucleus, where it binds to TCF/LEF transcription factors.⁹³ Once the transcriptional complexes are formed, downstream genes are activated to express themselves.⁹⁴ These β -catenin-induced genes promote rapid cell division by facilitating the transition from the G1 phase to the S phase of the cell cycle.⁹⁵ The G1/S phase transition requires CDK4/6 activity, which can be increased by upregulating β -catenin.⁹⁶ Additionally, β -catenin can prevent the production of genes linked to apoptosis, such as Bax and Bim, thereby inhibiting apoptosis.⁹⁷ Studies have

demonstrated that increased β -catenin accelerates HCC cell proliferation, invasion, and metastasis, and encourages tumor development.⁹⁸

Activation of the Wnt signaling pathway can sustain liver stem cells' capacity for self-renewal, allowing them to proliferate without differentiating into fully developed hepatocytes.⁹⁹ HCC may originate from these undifferentiated stem cells.¹⁰⁰ Several genes linked to stem cell properties can also be activated by the accumulation of β -catenin in the nucleus.¹⁰¹ Studies have indicated that HCC cells driven by the Wnt signaling pathway exhibit stem cell-like characteristics, including the ability to differentiate in multiple directions and self-renew.¹⁰² By upregulating the expression of genes related to the cell cycle, inhibiting apoptosis, and promoting HCC cell proliferation, the Wnt pathway enables HCC cells to survive and grow in harsh conditions.¹⁰³ The Wnt signaling pathway also promotes cell division by upregulating the production of cyclins and kinases, thereby maintaining the stem cell pool, activating DNA damage repair mechanisms, and helping HCC cells repair DNA damage, which supports their self-renewal ability.^{104,105}

Invasion and metastasis: The increased malignant biological behavior of HCC patients is a major cause of their high mortality rate. Wnt signaling pathway activation and the development of EMT are closely related.¹⁰⁶ EMT enables epithelial cells, such as liver cells, to shed their epithelial characteristics and acquire mesenchymal characteristics, such as the ability to migrate and invade. EMT is considered a key indicator of tumor cells' propensity to infiltrate.¹⁰⁷ The Wnt signaling pathway regulates the expression of N- and E-cadherin, which can further this process.¹⁰⁸ E-cadherin is a protein responsible for the adhesion of epithelial cells. The Wnt signaling pathway prevents the expression of E-cadherin by accumulating β -catenin, and its decreased expression leads to reduced intercellular adhesion, promoting EMT.¹⁰⁹ The β -catenin-TCF/LEF complex can activate mesenchymal markers such as vimentin, fibronectin, and N-cadherin. The upregulation of these proteins imparts interstitial properties to cells.^{110,111} The mesenchymal characteristics acquired during EMT facilitate the migration of HCC cells to surrounding tissues. Mesenchymal cells also exhibit enhanced invasion ability, allowing them to enter the blood circulation by passing through the vascular wall and basement membrane, thereby increasing the metastatic potential of HCC.¹¹² Additionally, HCC cells with mesenchymal characteristics enable epithelial cells to survive even in the absence of adhesion, inhibiting tumor cell apoptosis.¹¹³ HCC cells' ability to upregulate angiogenesis-related factors during EMT, such as vascular endothelial growth factor (VEGF), helps supply nutrients and oxygen for tumor development, potentially promoting the formation of tumor blood vessels.¹¹⁴ The Wnt signaling pathway can increase the expression of matrix metalloproteinase (MMP) genes, such as MMP-2 and MMP-9, and facilitate β -catenin's nuclear translocation and binding to the transcription factor TCF/LEF. These enzymes promote tumor cell motility and invasion by breaking down collagen and elastin, two components of the extracellular matrix (ECM). While MMP activity is negatively regulated by tissue inhibitors of metalloproteinases (TIMPs), MMPs are crucial for matrix disintegration.¹¹⁵ By promoting the breakdown of TIMPs, the Wnt signaling pathway can increase MMP activity and reduce ECM inhibition. In some cases, the Wnt signaling pathway may directly increase TIMP expression, but this typically occurs in conjunction with the degradation of MMPs to maintain proper ECM degradation.¹¹⁶

The Wnt signaling pathway can also increase integrin expression. Integrins bind to ECM components, enhancing cell

Table 1. Drugs acting on the Wnt/ β -catenin signaling pathway in HCC

Medicines	Action mechanism	Clinical stage	References
LGK974	Inhibition of Porcupine reduces the secretion of Wnt ligands	Clinical phase I	126
PRI-724	Inhibit the interaction between β -catenin and CBP, block the signal transduction	Clinical phase I	127
ICG-001	Inhibition of β -catenin binding to CBP blocks Wnt/ β -catenin pathway	Preclinical study/ clinical phase I	128
FH535	Inhibition of Wnt/ β -catenin and PPAR pathways	Preclinical study	129
XAV939	Inhibit Tankyrase, increase AXIN level and reduce the accumulation of β -catenin	Preclinical study	130
Vantictumab	Antibody drugs, targeting Frizzled receptors, block Wnt signaling	Preclinical study/ clinical phase I	131
DKK1	Blocking Wnt binding to Frizzled receptor and inhibiting Wnt pathway	Preclinical study	132
Niclosamide	Inhibition of Wnt/ β -catenin pathway reduces cell invasion ability.	Preclinical study	133
Salinomycin	By inhibiting the Wnt/ β -catenin pathway, it reduces the migration and invasion of cancer cells.	Preclinical study	134

HCC, hepatocellular carcinoma; DKK1, dickkopf wnt signaling pathway inhibitor 1; CBP, cyclic-AMP response element-binding protein; PPAR, peroxisome proliferator-activated receptor; AXIN, axis inhibition protein.

migration and invasion, and activating Rho family GTPases, including Rac1 and RhoA. These GTPases affect cell morphology and movement by regulating the polymerization and recombination of cytoskeletal proteins (such as actin and tubulin). These functions are primarily achieved by upregulating cell migration-related genes, such as MMPs, TIMPs, and cytoskeleton-related proteins.^{117,118} Moreover, Wnt signaling pathway activation may aid in modifying the HCC tumor microenvironment. In addition to promoting VEGF production, it can also support immunosuppression by inhibiting T cell activation and function, and by triggering the release of various cytokines and growth factors from tumor cells, such as platelet-derived growth factor, TGF- β , and fibroblast growth factor, all of which promote tumor invasion and metastasis.^{119,120} Furthermore, it can regulate the expression of cell adhesion molecules and chemokines, influencing immune cell recruitment and tumor cell infiltration. This suggests that tumor cells have an increased propensity to penetrate blood vessels and the lymphatic system, facilitating their entry into the blood circulation, infiltration, and metastasis.^{121–123}

Therapy

Chemotherapy is the mainstay of treatment for patients with advanced HCC. However, the emergence of targeted therapy has brought new hope for these patients. Compared with traditional chemotherapy methods, targeted therapy is more specific. It has less impact on the normal internal environment of the human body, fewer side effects, better drug compliance, and more obvious efficacy.¹²⁴ Because of the Wnt signaling pathway's crucial role in HCC, targeting it has become a feasible therapeutic strategy.¹²⁵ The related studies include Wnt signaling pathway inhibitors, downstream Wnt signaling pathway inhibitors, combination therapies targeting cell adhesion and migration, etc. (Table 1).^{126–134}

β -catenin inhibitors, which are further subdivided into GSK-3 β inhibitors and small molecule β -catenin inhibitors, are the most significant class of Wnt signaling pathway inhibitors.¹³⁵ β -catenin inhibitors in small molecules function in two ways. First, they bind directly to β -catenin, blocking its entry into the nucleus and activation of downstream genes. The second mechanism limits the activity of ubiquitinating enzymes (such as MMP7) to reduce β -catenin breakdown,

stabilizing it and lowering its activity.¹³⁶ Importantly, GSK-3 β inhibits the Wnt signaling pathway. Inhibiting its activity stabilizes β -catenin and leads to its accumulation in the nucleus. This indirect suppression of the Wnt signaling pathway has led to the development of GSK-3 β inhibitors.¹³⁷ In addition, Wnt competitive inhibitors are substances that competitively bind to Wnt receptors, preventing the activation of the Wnt signaling pathway. Currently, clinical trials for small molecule β -catenin inhibitors are underway, represented by Inducible Cytotoxic GPC3-001, which has entered the clinical trial stage. In patients with HCC, phase I clinical research has shown some anti-tumor activity and good tolerability; the phase II clinical trial is currently in progress to assess its safety and efficacy in more detail.¹³⁸ Wnt response inhibitors (IWR) are the main type of GSK-3 β inhibitor. Research on IWR-1 has attracted widespread attention and has been clinically tested in various cancer types, showing certain anti-tumor activity and potential for the treatment of HCC.¹³⁹ Representative Wnt competitive inhibitors include κ Ba (IkBa) analog inhibitors. IkBa analogs inhibit the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway by mimicking IkBa, which decreases IkB kinase activity and increases the expression of anti-apoptotic proteins (such as Bcl-2) while decreasing pro-apoptotic proteins (such as Bax), promoting apoptosis of HCC tumor cells.^{140,141} Clinical studies of related competitive inhibitors and antibodies in HCC are ongoing, and these drugs may provide new treatment options for HCC patients.

The downstream components of the Wnt signaling pathway are primarily responsible for obtaining, transmitting, and amplifying Wnt signals, including related receptors, TCF/LEF transcription factors, and effector molecules. An essential part of the Wnt signaling pathway is Frizzled (Fzd). Fzd receptors initiate signal transduction by attaching to Wnt ligands on the cell membrane. Fzd inhibitors interact with Frizzled receptors to prevent Wnt protein from binding, thus interrupting Wnt signal transduction, reducing downstream target gene transcription, lowering HCC cell survival and proliferation, and inhibiting tumor growth (Table 2).^{142–144} Inhibition of Fzd3 has been shown to suppress HCC cell proliferation and invasion while promoting tumor growth in animal models, providing a strong basis for further human studies.^{145,146} TCF/LEF transcription factors are crucial in the downstream

Table 2. Drugs targeting downstream effector molecules of Wnt signaling in HCC

Medicines	Action mechanism	Clinical stage	References
PNU-74654	It directly binds to β -catenin, prevents its formation with TCF complex, and reduces gene transcription	Preclinical study	142
MSAB	Inhibition of β -catenin-mediated transcriptional activity reduces Wnt signaling downstream gene expression	Preclinical study	143
PBP(Pyrvinium pamoate)	By interfering with the Wnt signaling pathway and inhibiting the activity of β -catenin, the transcription of downstream genes is inhibited	Preclinical study	144

HCC, hepatocellular carcinoma; MSAB, methylation-specific antibody; TCF, T-cell factor.

Wnt signaling cascade. Inhibitors of TCF/LEF have the potential to restrict the expression of Wnt target genes by blocking the interaction between β -catenin and TCF/LEF, disrupting the Wnt signaling pathway and decreasing tumor cell proliferation and survival.¹⁴⁷ Specific inhibitors of TCF/LEF include BAY 11-7082. Recent research has demonstrated that BAY 11-7082, in conjunction with CDK4/6 inhibitors, is beneficial against a range of malignancies, including HCC, in animal trials.¹⁴⁸ Furthermore, studies have shown a negative correlation between tumor growth and the production of WISP1, a downstream effector of the Wnt signaling pathway. WISP1 stops HCC progression by negatively regulating β -catenin/TCF/LEF signaling, suggesting a potential therapeutic avenue for targeting Wnt downstream effector molecules to halt HCC progression.¹⁴⁹

MMPs are important players in the Wnt signaling pathway, stimulating HCC tumor cells' adhesion, motility, and invasion. Inhibition of MMPs can reduce these processes and prevent premature metastasis of tumor cells.¹⁵⁰ MMP inhibitors directly block the activity of MMPs through chemical structures or biological pathways, reducing ECM degradation and regulating MMP gene expression or protein synthesis to lower MMP production.¹⁵¹ The current research on MMP inhibitors focuses on Batimastat (BB-94) and Marimastat (BB-2516).¹⁵² MMP inhibitors have been demonstrated in several clinical trials to reduce HCC tumor invasion and improve prognosis. Studies have shown that BB-94 can prevent HCC cells from migrating and inhibit angiogenesis in the tumor vascular system. Furthermore, the tumor blood vessels that are prevented from growing cannot supply enough oxygen and nutrients for the remaining HCC cells.¹⁵³ MMP inhibitors target MMPs overexpressed in the tissues and cells of hepatocellular carcinoma. After years of research, many clinical drugs have been produced, such as Bamalabaster, Novartis, BAY12-9566, AG-3340, OPB-3206, KBR07785, and KBR-8301.^{154–156} Cell adhesion molecules (CAMs) are important for mediating interactions between cells or between cells and the extracellular matrix.¹⁵⁷ CAM modulators can affect CAM content and activation pathways, influencing tumor cell adhesion, migration, and invasion (Table 3).^{158–175} Various types of CAM modulators include E-cadherin agonists, which can enhance intercellular adhesion and reduce tumor cell invasion by promoting E-cadherin expression and function.¹⁷⁶ SMADS agonists can initiate SMADS phosphorylation and nuclear translocation through the TGF- β /SMAD signaling pathway. By binding to the E-cadherin promoter region and promoting E-cadherin production, SMADS interacts with β -catenin to improve cell adhesion and Wnt signaling. Studies have indicated that interferon- α and TGF- β 1 may effectively block the development of the β -catenin/TCF4/Smads complex, suggesting that these molecules could be employed as potential therapeutics for HCC patients.^{177,178} Integrin modulators inhibit integrins from binding to ligands, reducing cell adhesion and migration. Examples include antibody-cou-

pled drugs including ramucirumab and atezolizumab, which can bind integrins and target tumor cells.^{179,180} Cetuximab, a small molecule integrin blocker, may be particularly effective in preventing advanced HCC by obstructing the interaction between integrins and EGFR.¹⁸¹

Several therapeutic modalities related to the Wnt signaling pathway, including immune checkpoint inhibitors and CAR-T cell therapy, are now in the preclinical or animal experimental stages, in addition to the previously mentioned treatment strategies.^{182,183} The combination of Wnt signaling pathway-related treatments with other therapeutic measures (such as immunotherapy, VEGF inhibitor therapy, etc.) can improve efficacy, eliminate the side effects of monotherapy, and reduce drug resistance.¹⁸⁴ Targeting the Wnt signaling pathway holds great potential in clinical practice, but it also faces several challenges. Therapeutic approaches targeting the Wnt pathway may lead to non-specific off-target effects, affecting the function of normal cells and tissues, particularly in the liver, intestine, and bones.¹⁸⁵ Although targeting the Wnt pathway has shown promising efficacy *in vitro* and in animal models, drug resistance is a common issue in clinical applications. Activation of the Wnt signaling pathway involves multiple regulatory mechanisms, including the stability of β -catenin, upstream Frizzled receptors, and downstream transcription factors. The complexity of these regulatory processes allows tumor cells to bypass targeted therapies in various ways.¹⁸⁶ Additionally, abnormal stability or nuclear translocation of β -catenin may be maintained through mutations or other pathways, reducing the effectiveness of drugs.¹⁸⁷ The regulation of the Wnt pathway is highly complex, involving multiple key molecules, and different types of tumors may depend on different Wnt signaling subtypes, which complicates targeted Wnt therapy and introduces issues of tumor heterogeneity.¹⁸⁸ The Wnt signaling pathway's high degree of intricacy and variety in regulation is a result of its numerous interactions with other signaling pathways. Accurate targeting is extremely challenging, which highlights the significance of combination therapy. Due to the Wnt pathway's intricate role in the human body, therapeutic techniques for it are currently being developed slowly and require further research.

Conclusions

The initiation, development, and metastasis of HCC are closely dependent on the Wnt signaling pathway. One mechanism driving aberrant activation of this pathway is the production of excess Wnt ligands, which leads to mutations in Wnt receptors or downstream effector molecules, such as β -catenin, and the loss of Wnt pathway inhibitors. However, despite increasing research suggesting the potential for targeted therapies that modulate the Wnt signaling pathway, several challenges remain. The complexity of the Wnt pathway, the diversity of cancer types, and the issue of drug re-

Table 3. Drugs targeting cell adhesion and migration in the Wnt signaling pathway in HCC

Medicines	Action mechanism	Clinical stage	References
Batimastat (BB-94)	Broad-spectrum MMP inhibitors inhibit MMP-1, MMP-2, MMP-9, etc., and reduce tumor invasion and migration	Clinical stage I/II	158
Marimastat (BB-2516)	Broad-spectrum MMP inhibitors inhibit MMP-1, MMP-2, MMP-9, etc., and reduce tumor invasion and migration	Clinical Phase II	159
Tanomastat (BAY 12-9566)	MMP inhibitors, targeting MMP-2 and MMP-9, inhibit the degradation of extracellular matrix and prevent tumor metastasis	Clinical Phase II	160
TIMP-2	Endogenous MMP inhibitors directly inhibit MMP-2 activity and reduce matrix degradation and tumor invasion	Preclinical study	161
Doxycycline	Antibiotics, non-specific MMP inhibitors, inhibit MMP-2 and MMP-9, reduce tumor invasion and metastasis	Preclinical study/ clinical phase I	162
Ilomastat (GM6001)	Broad-spectrum MMP inhibitors, inhibit MMP-1, MMP-2, MMP-9, reduce extracellular matrix degradation	Preclinical study	163
AG3340 (Prinomastat)	Selective MMP inhibitors, mainly inhibit MMP-2 and MMP-9, reduce extracellular matrix degradation and invasion	Clinical phase I	164
ONO-4817	Selective inhibition of MMP-2 and MMP-9 reduced tumor cell invasion and matrix degradation	Preclinical study	165
Minocycline	Inhibit the activity of MMP-2 and MMP-9, reduce matrix degradation and tumor cell migration	Preclinical study	166
E7820	Inhibit the expression of integrin $\alpha 2$, reduce tumor cell adhesion and angiogenesis	Clinical stage I / II	167
Volociximab	Targeting integrin $\alpha 5\beta 1$ prevents the binding of tumor cells to the extracellular matrix and inhibits tumor invasion	Clinical Phase II	168
Etaracizumab (Abegrin)	Targeting integrin $\alpha v\beta 3$ reduces tumor cell adhesion and angiogenesis, and inhibits tumor growth and metastasis	Clinical Phase II	169
Cilengitide	Inhibition of integrin $\alpha v\beta 3$ and $\alpha v\beta 5$ inhibits tumor angiogenesis and tumor cell invasion.	Clinical Phase II	170
Simtuzumab (GS-6624)	Anti-LOXL2 (lysyl oxidase-like protein 2) antibody prevents extracellular matrix remodeling and reduces tumor invasion	Clinical Phase II	171
Disulfiram	It inhibits the expression of cell adhesion molecules (such as N-cadherin) and prevents cancer cell migration and invasion	Preclinical study	172
ADH-1	Targeting N-cadherin inhibits intercellular adhesion and reduces invasion and metastasis of tumor cells	Preclinical study	173
Galunisertib (LY2157299)	TGF- β inhibitors inhibit the expression of adhesion molecules and reduce tumor cell invasion and metastasis	Clinical Phase II	174
Metformin	Indirectly inhibit the expression of cell adhesion molecules, reduce the invasion ability of tumor cells	Preclinical study	175

HCC, hepatocellular carcinoma; MMP, matrix metalloproteinase; TGF- β , transforming growth factor β .

sistance are major obstacles. These factors contribute to the absence of clinically available Wnt signaling inhibitors, making effective treatment difficult.

In conclusion, while our understanding of the Wnt signaling pathway in HCC is still evolving, there is hope for the development of more precise targeted therapies in the future. These therapies would aim to control toxic side effects while providing effective treatment. Additionally, personalized treatment plans based on a patient's specific tumor genome and clinical features may become feasible. Identifying biomarkers to select patients who are most likely to benefit from Wnt-targeted therapies represents an exciting direction for future research in this area.

Funding

This research was funded by the National Natural Science Foundation of China (82472743; 82300921); the Beijing

Xisike Clinical Oncology Research Foundation (Y-QL202201-0020); the Beijing Science and Technology Innovation Medical Development Foundation (KC2023-JX-0186-FM046); the Anhui Engineering Technology Research Center of Biochemical Pharmaceutical (Bengbu Medical University)(2024SYKFD01); the Opening Project of the Scientific and Technological Innovation Major Base of Guangxi (2022-36-Z05-GX-SWBX202201); the Key Laboratory of Clinical Laboratory Diagnosis and Translational Research of Zhejiang Province (2022E10022); the Opening Project of Fujian Provincial Key Laboratory of Tumor Biotherapy (FJZL2023001); the Opening Project of Fujian Provincial Key Laboratory of Translational Cancer Medicine (TCM2024-3); the Opening Project of Fujian Provincial Key Laboratory of Innovative Drug Target Research (FJ-YW-2024KF02); the Thematic Research Support Scheme of the State Key Laboratory of Liver Research, The University of Hong Kong (SKLLR/TRSS/2022/08); the Chen Xiaoping Foundation for the Development of Science

and Technology of Hubei Province (CXPJH123003-005); the Postgraduate Research & Practice Innovation Program of Harbin Medical University (YJSCX2024-110HYD); the Construction Project of Shanghai Key Laboratory of Molecular Imaging (18DZ2260400).

Conflict of interest

JWPY has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2021. The other authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (ZL, SL, ZW, JZ, ZH, JL, HB), writing of the manuscript (ZL, SL), revision (JWPY, YX). All authors have read and agreed to the published version of the manuscript.

References

- Wong CM, Tsang FH, Ng IO. Non-coding RNAs in hepatocellular carcinoma: molecular functions and pathological implications. *Nat Rev Gastroenterol Hepatol* 2018;15(3):137–151. doi:10.1038/nrgastro.2017.169, PMID:29317776.
- Affo S, Yu LX, Schwabe RF. The Role of Cancer-Associated Fibroblasts and Fibrosis in Liver Cancer. *Annu Rev Pathol* 2017;12:153–186. doi:10.1146/annurev-pathol-052016-100322, PMID:27959632.
- Bakrania A, Zheng G, Bhat M. Nanomedicine in Hepatocellular Carcinoma: A New Frontier in Targeted Cancer Treatment. *Pharmaceutics* 2021;14(1):41. doi:10.3390/pharmaceutics14010041, PMID:35056937.
- Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362(9399):1907–1917. doi:10.1016/S0140-6736(03)14964-1, PMID:14667750.
- Chakraborty E, Sarkar D. Emerging Therapies for Hepatocellular Carcinoma (HCC). *Cancers (Basel)* 2022;14(11):2798. doi:10.3390/cancers14112798, PMID:35681776.
- Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019;16(7):411–428. doi:10.1038/s41575-019-0145-7, PMID:31028350.
- Thandra KC, Barsouk A, Saginala K, Aluru JS, Rawla P, Barsouk A. Epidemiology of non-alcoholic fatty liver disease and risk of hepatocellular carcinoma progression. *Clin Exp Hepatol* 2020;6(4):289–294. doi:10.5114/ceh.2020.102153, PMID:33511275.
- Vogel A, Martinelli E, ESMO Guidelines Committee. Updated treatment recommendations for hepatocellular carcinoma (HCC) from the ESMO Clinical Practice Guidelines. *Ann Oncol* 2021;32(6):801–805. doi:10.1016/j.annonc.2021.02.014, PMID:33716105.
- Jemal A, Ward EM, Johnson CJ, Cronin KA, Ma J, Ryerson B, *et al*. Annual Report to the Nation on the Status of Cancer, 1975–2014, Featuring Survival. *J Natl Cancer Inst* 2017;109(9):dix030. doi:10.1093/jnci/dix030, PMID:28376154.
- Zhou Y, Xu J, Luo H, Meng X, Chen M, Zhu D. Wnt signaling pathway in cancer immunotherapy. *Cancer Lett* 2022;525:84–96. doi:10.1016/j.canlet.2021.10.034, PMID:34740608.
- Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, *et al*. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. *Mol Cancer* 2022;21(1):144. doi:10.1186/s12943-022-01616-7, PMID:35836256.
- Xu X, Zhang M, Xu F, Jiang S. Wnt signaling in breast cancer: biological mechanisms, challenges and opportunities. *Mol Cancer* 2020;19(1):165. doi:10.1186/s12943-020-01276-5, PMID:33234169.
- Chaisaingmongkol J, Budhu A, Dang H, Rabibhadana S, Pupacdi B, Kwon SM, *et al*. Common Molecular Subtypes Among Asian Hepatocellular Carcinoma and Cholangiocarcinoma. *Cancer Cell* 2017;32(1):57–70.e3. doi:10.1016/j.ccell.2017.05.009, PMID:28648284.
- Wang M, Guo H, Zhang B, Shang Y, Zhang S, Liu X, *et al*. Transcription factors-related molecular subtypes and risk prognostic model: exploring the immunogenicity landscape and potential drug targets in hepatocellular carcinoma. *Cancer Cell Int* 2024;24(1):9. doi:10.1186/s12935-023-03185-1, PMID:38178084.
- Yang S, Zheng L, Li L, Zhang J, Wang J, Zhao H, *et al*. Integrative multiomics analysis identifies molecular subtypes and potential targets of hepatocellular carcinoma. *Clin Transl Med* 2024;14(6):e1727. doi:10.1002/ctm2.1727, PMID:38804617.
- Zeng C, Zhang L, Luo C, Yang C, Huang X, Fan L, *et al*. A stratification model of hepatocellular carcinoma based on expression profiles of cells in the tumor microenvironment. *BMC Cancer* 2022;22(1):613. doi:10.1186/s12885-022-09647-5, PMID:35659630.
- Nusse R, Clevers H. Wnt/ β -Catenin Signaling, Disease, and Emerging Therapeutic Modalities. *Cell* 2017;169(6):985–999. doi:10.1016/j.cell.2017.05.016, PMID:28575679.
- Bender W, Peifer M. Oncogenes take wing. *Cell* 1987;50(4):519–520. doi:10.1016/0092-8674(87)90023-7, PMID:3111718.
- Sharma RP, Chopra VL. Effect of the Wingless (wg1) mutation on wing and haltere development in *Drosophila melanogaster*. *Dev Biol* 1976;48(2):461–465. doi:10.1016/0012-1606(76)90108-1, PMID:8151114.
- Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, *et al*. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 1991;253(5020):665–669. doi:10.1126/science.1651563, PMID:1651563.
- Pez F, Lopez A, Kim M, Wands JR, Caron de Fromental C, Merle P. Wnt signaling and hepatocarcinogenesis: molecular targets for the development of innovative anticancer drugs. *J Hepatol* 2013;59(5):1107–1117. doi:10.1016/j.jhep.2013.07.001, PMID:23835194.
- Chan KK, Lo RC. Deregulation of Frizzled Receptors in Hepatocellular Carcinoma. *Int J Mol Sci* 2018;19(1):313. doi:10.3390/ijms19010313, PMID:29361730.
- Yao H, Ashihara E, Maekawa T. Targeting the Wnt/ β -catenin signaling pathway in human cancers. *Expert Opin Ther Targets* 2011;15(7):873–887. doi:10.1517/14728222.2011.577418, PMID:21486121.
- Luga V, Zhang L, Vitoria-Petit AM, Ogunjimi AA, Inanlou MR, Chiu E, *et al*. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell* 2012;151(7):1542–1556. doi:10.1016/j.cell.2012.11.024, PMID:23260141.
- Qiu JJ, Sun SG, Tang XY, Lin YY, Hua KQ. Extracellular vesicular Wnt7b mediates HPV E6-induced cervical cancer angiogenesis by activating the β -catenin signaling pathway. *J Exp Clin Cancer Res* 2020;39(1):260. doi:10.1186/s13046-020-01745-1, PMID:33234148.
- Taelman VF, Dobrowolski R, Plouhinec JL, Fuentealba LC, Vorwald PP, Gumper I, *et al*. Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. *Cell* 2010;143(7):1136–1148. doi:10.1016/j.cell.2010.11.034, PMID:21183076.
- Petővári G, Tóth G, Turiák L, Kiss AL, Pálóczi K, Sebestyén A, *et al*. Dynamic Interplay in Tumor Ecosystems: Communication between Hepatoma Cells and Fibroblasts. *Int J Mol Sci* 2023;24(18):13996. doi:10.3390/ijms241813996, PMID:37762298.
- Wu Y, Li H, Long Y, Zhang Z, Zhang F, Pan R, *et al*. Epigenetic Suppression of miR-137 Induces RNF4 Expression, Facilitating Wnt Signaling in Colorectal Cancer. *Mol Carcinog* 2024. doi:10.1002/mc.23859, PMID:39630054.
- Lin C, Wu J, Wang Z, Xiang Y. Long non-coding RNA LNC-POTEM-4 promotes HCC progression via the LNC-POTEM-4/miR-149-5p/Wnt4 signaling axis. *Cell Signal* 2024;124:111412. doi:10.1016/j.cellsig.2024.111412, PMID:39278454.
- Martin-Medina A, Lehmann M, Burgoyne O, Hermann S, Baarsma HA, Wagner DE, *et al*. Increased Extracellular Vesicles Mediate WNT5A Signaling in Idiopathic Pulmonary Fibrosis. *Am J Respir Crit Care Med* 2018;198(12):1527–1538. doi:10.1164/rccm.201708-1580OC, PMID:30044642.
- Han SI, Lim SC. Expression and Prognostic Significance of CDK8 and β -Catenin in Hepatocellular Carcinoma. *In Vivo* 2020;34(3):1387–1394. doi:10.21873/invivo.11918, PMID:32354935.
- Li YH, Yang SL, Zhang GF, Wu JC, Gong LL, Ming-Zhong, *et al*. Mefloquine targets β -catenin pathway and thus can play a role in the treatment of liver cancer. *Microb Pathog* 2018;118:357–360. doi:10.1016/j.micpath.2018.03.042, PMID:29578061.
- Xu C, Xu Z, Zhang Y, Evert M, Calvisi DF, Chen X. β -Catenin signaling in hepatocellular carcinoma. *J Clin Invest* 2022;132(4):e154515. doi:10.1172/JCI154515, PMID:35166233.
- de Man SMA, van Amerongen R. Zooming in on the WNT/CTNNB1 Destruction Complex: Functional Mechanistic Details with Implications for Therapeutic Targeting. *Handb Exp Pharmacol* 2021;269:137–173. doi:10.1007/164_2021_522, PMID:34486095.
- Tran FH, Zheng JJ. Modulating the wnt signaling pathway with small molecules. *Protein Sci* 2017;26(4):650–661. doi:10.1002/pro.3122, PMID:28120389.
- Wu S, Liu Z, Chen J, Ma T, Wang L, Huang T, *et al*. PORCN promotes hepatocellular carcinogenesis via Wnt/ β -catenin-dependent manner. *Transl Cancer Res* 2023;12(7):1703–1714. doi:10.21037/tcr-23-191, PMID:37588740.
- Lv D, Chen L, Du L, Zhou L, Tang H, Tang H. Emerging Regulatory Mechanisms Involved in Liver Cancer Stem Cell Properties in Hepatocellular Carcinoma. *Front Cell Dev Biol* 2021;9:691410. doi:10.3389/fcell.2021.691410, PMID:34368140.
- Fan J, Wei Q, Liao J, Zou Y, Song D, Xiong D, *et al*. Noncanonical Wnt signaling plays an important role in modulating canonical Wnt-regulated stemness, proliferation and terminal differentiation of hepatic progenitors. *Oncotarget* 2017;8(16):27105–27119. doi:10.18632/oncotarget.15637, PMID:28404920.
- He K, Gan WJ. Wnt/ β -Catenin Signaling Pathway in the Development and Progression of Colorectal Cancer. *Cancer Manag Res* 2023;15:435–448. doi:10.2147/CMAR.S411168, PMID:37250384.
- Li VS, Ng SS, Boersema PJ, Low TY, Karthaus WR, Gerlach JP, *et al*. Wnt signaling through inhibition of β -catenin degradation in an intact Axin1 complex. *Cell* 2012;149(6):1245–1256. doi:10.1016/j.cell.2012.05.002, PMID:22682247.
- Polakis P. Wnt signaling in cancer. *Cold Spring Harb Perspect Biol* 2012;4(5):a008052. doi:10.1101/cshperspect.a008052, PMID:22438566.
- Niehrs C. The complex world of Wnt receptor signalling. *Nat Rev Mol Cell Biol* 2012;13(12):767–779. doi:10.1038/nrm3470, PMID:23151663.
- Cadigan KM, Waterman ML. TCF/LEFs and Wnt signaling in the nucleus. *Cold Spring Harb Perspect Biol* 2012;4(11):a007906. doi:10.1101/cshperspect.a007906, PMID:23024173.
- Steinhart Z, Angers S. Wnt signaling in development and tissue homeostasis. *Development* 2018;145(11):dev146589. doi:10.1242/dev.146589,

- PMID:29884654.
- [45] Kretzschmar K, Clevers H. Wnt/ β -catenin signaling in adult mammalian epithelial stem cells. *Dev Biol* 2017;428(2):273–282. doi:10.1016/j.ydbio.2017.05.015, PMID:28526587.
 - [46] Thompson MD, Monga SP. WNT/ β -catenin signaling in liver health and disease. *Hepatology* 2007;45(5):1298–1305. doi:10.1002/hep.21651, PMID:17464972.
 - [47] Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene* 2017;36(11):1461–1473. doi:10.1038/onc.2016.304, PMID:27617575.
 - [48] VanderVorst K, Dreyer CA, Konopelski SE, Lee H, Ho HH, Carraway KL 3rd. Wnt/PCP Signaling Contribution to Carcinoma Collective Cell Migration and Metastasis. *Cancer Res* 2019;79(8):1719–1729. doi:10.1158/0008-5472.CAN-18-2757, PMID:30952630.
 - [49] Pataki CA, Couchman JR, Brábek J. Wnt Signaling Cascades and the Roles of Syndecan Proteoglycans. *J Histochem Cytochem* 2015;63(7):465–480. doi:10.1369/0022155415586961, PMID:25910817.
 - [50] Farb MG, Karki S, Park SY, Saggese SM, Carmine B, Hess DT, *et al*. WNT5A-JNK regulation of vascular insulin resistance in human obesity. *Vasc Med* 2016;21(6):489–496. doi:10.1177/1358863X16666693, PMID:27688298.
 - [51] Zhu Y, Li X. Advances of Wnt Signalling Pathway in Colorectal Cancer. *Cells* 2023;12(3):447. doi:10.3390/cells12030447, PMID:36766788.
 - [52] Molkenin JD. Calcineurin-NFAT signaling regulates the cardiac hypertrophic response in coordination with the MAPKs. *Cardiovasc Res* 2004;63(3):467–475. doi:10.1016/j.cardiores.2004.01.021, PMID:15276472.
 - [53] Lang CMR, Chan CK, Veltri A, Lien WH. Wnt Signaling Pathways in Keratinocyte Carcinomas. *Cancers (Basel)* 2019;11(9):E1216. doi:10.3390/cancers11091216, PMID:31438551.
 - [54] De A. Wnt/Ca²⁺ signaling pathway: a brief overview. *Acta Biochim Biophys Sin (Shanghai)* 2011;43(10):745–756. doi:10.1093/abbs/gmr079, PMID:21903638.
 - [55] Chen Y, Chen Z, Tang Y, Xiao Q. The involvement of noncanonical Wnt signaling in cancers. *Biomed Pharmacother* 2021;133:110946. doi:10.1016/j.biopha.2020.110946, PMID:33212376.
 - [56] Wang W, Smits R, Hao H, He C. Wnt/ β -Catenin Signaling in Liver Cancers. *Cancers (Basel)* 2019;11(7):926. doi:10.3390/cancers11070926, PMID:31269694.
 - [57] Khalaf AM, Fuentes D, Morshid AI, Burke MR, Kaseb AO, Hassan M, *et al*. Role of Wnt/ β -catenin signaling in hepatocellular carcinoma, pathogenesis, and clinical significance. *J Hepatocell Carcinoma* 2018;5:61–73. doi:10.2147/JHC.S156701, PMID:29984212.
 - [58] Lin J, Lu W, Huang B, Yang W, Wang X. The role of tissue-derived extracellular vesicles in tumor microenvironment. *Tissue Cell* 2024;89:102470. doi:10.1016/j.tice.2024.102470, PMID:39002287.
 - [59] Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, *et al*. Role of HIF-1 α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 1998;394(6692):485–490. doi:10.1038/28867, PMID:9697772.
 - [60] Sun X, Hu F, Hou Z, Chen Q, Lan J, Luo X, *et al*. SIX4 activates Akt and promotes tumor angiogenesis. *Exp Cell Res* 2019;383(1):111495. doi:10.1016/j.yexcr.2019.111495, PMID:31301290.
 - [61] Monga SP. β -Catenin Signaling and Roles in Liver Homeostasis, Injury, and Tumorigenesis. *Gastroenterology* 2015;148(7):1294–1310. doi:10.1053/j.gastro.2015.02.056, PMID:25747274.
 - [62] Gougelet A, Torre C, Veber P, Sartor C, Bachelot L, Denechaud PD, *et al*. T-cell factor 4 and β -catenin chromatin occupancies pattern zonal liver metabolism in mice. *Hepatology* 2014;59(6):2344–2357. doi:10.1002/hep.26924, PMID:24214913.
 - [63] Gougelet A, Colnot S. A Complex Interplay between Wnt/ β -Catenin Signaling and the Cell Cycle in the Adult Liver. *Int J Hepatol* 2012;2012:816125. doi:10.1155/2012/816125, PMID:22973520.
 - [64] Sanceau J, Poupel J, Joubel C, Lagoutte I, Caruso S, Pinto S, *et al*. DLK1/DIO3 locus upregulation by a β -catenin-dependent enhancer drives cell proliferation and liver tumorigenesis. *Mol Ther* 2024;32(4):1125–1143. doi:10.1016/j.ymthe.2024.01.036, PMID:38311851.
 - [65] Ning X, Shi Z, Liu X, Zhang A, Han L, Jiang K, *et al*. DNMT1 and EZH2 mediated methylation silences the microRNA-200b/a/429 gene and promotes tumor progression. *Cancer Lett* 2015;359(2):198–205. doi:10.1016/j.canlet.2015.01.005, PMID:25595591.
 - [66] Elmileik H, Paterson AC, Kew MC. Beta-catenin mutations and expression, 249serine p53 tumor suppressor gene mutation, and hepatitis B virus infection in southern African Blacks with hepatocellular carcinoma. *J Surg Oncol* 2005;91(4):258–263. doi:10.1002/jso.20304, PMID:16121349.
 - [67] Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Reboursou S, *et al*. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015;47(5):505–511. doi:10.1038/ng.3252, PMID:25822088.
 - [68] Colnot S, Decaens T, Niwa-Kawakita M, Godard C, Hamard G, Kahn A, *et al*. Liver-targeted disruption of Apc in mice activates beta-catenin signaling and leads to hepatocellular carcinomas. *Proc Natl Acad Sci U S A* 2004;101(49):17216–17221. doi:10.1073/pnas.0404761101, PMID:15563600.
 - [69] Taniguchi K, Roberts LR, Aderca IN, Dong X, Qian C, Murphy LM, *et al*. Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. *Oncogene* 2002;21(31):4863–4871. doi:10.1038/sj.onc.1205591, PMID:12101426.
 - [70] Zhang XZ, Mo XC, Wang ZT, Sun R, Sun DQ. N-glycosylation of Wnt3 regulates the progression of hepatocellular carcinoma by affecting Wnt/ β -catenin signal pathway. *World J Gastrointest Oncol* 2024;16(6):2769–2780. doi:10.4251/wjgo.v16.i6.2769, PMID:38994173.
 - [71] Jones SE, Jomary C. Secreted Frizzled-related proteins: searching for relationships and patterns. *Bioessays* 2002;24(9):811–820. doi:10.1002/bies.10136, PMID:12210517.
 - [72] Liu HY, Sun XJ, Xiu SY, Zhang XY, Wang ZQ, Gu YL, *et al*. Frizzled receptors (FZDs) in Wnt signaling: potential therapeutic targets for human cancers. *Acta Pharmacol Sin* 2024;45(8):1556–1570. doi:10.1038/s41401-024-01270-3, PMID:38632318.
 - [73] Takagi H, Sasaki S, Suzuki H, Toyota M, Maruyama R, Nojima M, *et al*. Frequent epigenetic inactivation of SFRP genes in hepatocellular carcinoma. *J Gastroenterol* 2008;43(5):378–389. doi:10.1007/s00535-008-2170-0, PMID:18592156.
 - [74] He S, Tang S. WNT/ β -catenin signaling in the development of liver cancers. *Biomed Pharmacother* 2020;132:110851. doi:10.1016/j.biopha.2020.110851, PMID:33080466.
 - [75] Li C, Furth EE, Rustgi AK, Klein PS. When You Come to a Fork in the Road, Take It: Wnt Signaling Activates Multiple Pathways through the APC/Axin/GSK-3 Complex. *Cells* 2023;12(18):2256. doi:10.3390/cells12182256, PMID:37759479.
 - [76] Lu G, Lin J, Song G, Chen M. Prognostic significance of CTNNB1 mutation in hepatocellular carcinoma: a systematic review and meta-analysis. *Aging (Albany NY)* 2023;15(18):9759–9778. doi:10.18632/aging.205047, PMID:37733676.
 - [77] Jung YS, Stratton SA, Lee SH, Kim MJ, Jun S, Zhang J, *et al*. TMEM9-v-ATPase Activates Wnt/ β -Catenin Signaling Via APC Lysosomal Degradation for Liver Regeneration and Tumorigenesis. *Hepatology* 2021;73(2):776–794. doi:10.1002/hep.31305, PMID:32380568.
 - [78] Zhang C, Li J, Huang T, Duan S, Dai D, Jiang D, *et al*. Meta-analysis of DNA methylation biomarkers in hepatocellular carcinoma. *Oncotarget* 2016;7(49):81255–81267. doi:10.18632/oncotarget.13221, PMID:27835605.
 - [79] Chen J, Gindoff JA, Su X. Immunomodulatory TGF- β Signaling in Hepatocellular Carcinoma. *Trends Mol Med* 2019;25(11):1010–1023. doi:10.1016/j.molmed.2019.06.007, PMID:31353124.
 - [80] Zhu W, Qin L. GOLM1-regulated EGFR/RTK recycling is a novel target for combating HCC metastasis. *Sci China Life Sci* 2017;60(1):98–101. doi:10.1007/s11427-016-0311-x, PMID:27858335.
 - [81] Timperi E, Barnaba V. Viral Hepatitis, Inflammation and Tumour Microenvironment. *Adv Exp Med Biol* 2020;1263:25–43. doi:10.1007/978-3-030-44518-8_3, PMID:32588321.
 - [82] da Motta Girardi D, Correa TS, Crosara Teixeira M, Dos Santos Fernandes G. Hepatocellular Carcinoma: Review of Targeted and Immune Therapies. *J Gastrointest Cancer* 2018;49(3):227–236. doi:10.1007/s12029-018-0121-4, PMID:29806062.
 - [83] Tigue ML, Loberg MA, Goettel JA, Weiss WA, Lee E, Weiss VL. Wnt Signaling in the Phenotype and Function of Tumor-Associated Macrophages. *Cancer Res* 2023;83(1):3–11. doi:10.1158/0008-5472.CAN-22-1403, PMID:36214645.
 - [84] Aoki T, Nishida N, Kurebayashi Y, Sakai K, Morita M, Chishina H, *et al*. Two Distinct Characteristics of Immune Microenvironment in Human Hepatocellular Carcinoma with Wnt/ β -Catenin Mutations. *Liver Cancer* 2024;13(3):285–305. doi:10.1159/000533818, PMID:38894812.
 - [85] Anna CH, Iida M, Sills RC, Devereux TR. Expression of potential beta-catenin targets, cyclin D1, c-Jun, c-Myc, E-cadherin, and EGFR in chemically induced hepatocellular neoplasms from B6C3F1 mice. *Toxicol Appl Pharmacol* 2003;190(2):135–145. doi:10.1016/s0041-008x(03)00170-4, PMID:12878043.
 - [86] Doumpas N, Lampart F, Robinson MD, Lentini A, Nestor CE, Cantù C, *et al*. TCF/LEF dependent and independent transcriptional regulation of Wnt/ β -catenin target genes. *EMBO J* 2019;38(2):e98873. doi:10.15252/emboj.201798873, PMID:30425074.
 - [87] Davidson G. The cell cycle and Wnt. *Cell Cycle* 2010;9(9):1667–1668. doi:10.4161/cc.9.9.11595, PMID:20404508.
 - [88] Zhang JH, Jiao LY, Li TJ, Zhu YY, Zhou JW, Tian J. GSK-3 β suppresses HCC cell dissociation in vitro by upregulating epithelial junction proteins and inhibiting Wnt/ β -catenin signaling pathway. *J Cancer* 2017;8(9):1598–1608. doi:10.7150/jca.18744, PMID:28775779.
 - [89] Lu FI, Sun YH, Wei CY, Thisse C, Thisse B. Tissue-specific derepression of TCF/LEF controls the activity of the Wnt/ β -catenin pathway. *Nat Commun* 2014;5:5368. doi:10.1038/ncomms6368, PMID:25371059.
 - [90] MacDonald BT, He X. Frizzled and LRP5/6 receptors for Wnt/ β -catenin signaling. *Cold Spring Harb Perspect Biol* 2012;4(12):a007880. doi:10.1101/cshperspect.a007880, PMID:23209147.
 - [91] Biswas P, Canosa S, Schoenfeld D, Schoenfeld J, Li P, Cheas LC, *et al*. PECAM-1 affects GSK-3 β -mediated beta-catenin phosphorylation and degradation. *Am J Pathol* 2006;169(1):314–324. doi:10.2353/ajpath.2006.051112, PMID:16816383.
 - [92] Lin J, Song T, Li C, Mao W. GSK-3 β in DNA repair, apoptosis, and resistance of chemotherapy, radiotherapy of cancer. *Biochim Biophys Acta Mol Cell Res* 2020;1867(5):118659. doi:10.1016/j.bbamcr.2020.118659, PMID:31978503.
 - [93] Kim W, Kim M, Jho EH. Wnt/ β -catenin signalling: from plasma membrane to nucleus. *Biochem J* 2013;450(1):9–21. doi:10.1042/BJ20121284, PMID:23343194.
 - [94] Novak A, Dedhar S. Signaling through beta-catenin and Lef/Tcf. *Cell Mol Life Sci* 1999;56(5-6):523–537. doi:10.1007/s000180050449, PMID:11212302.
 - [95] Hume S, Dianov GL, Ramadan K. A unified model for the G1/S cell cycle transition. *Nucleic Acids Res* 2020;48(22):12483–12501. doi:10.1093/nar/gkaa1002, PMID:33166394.
 - [96] Bertoli C, Skotheim JM, de Bruijn RA. Control of cell cycle transcription during G1 and S phases. *Nat Rev Mol Cell Biol* 2013;14(8):518–528. doi:10.1038/nrm3629, PMID:23877564.
 - [97] Huang M, Wang Y, Sun D, Zhu H, Yin Y, Zhang W, *et al*. Identification

- of genes regulated by Wnt/beta-catenin pathway and involved in apoptosis via microarray analysis. *BMC Cancer* 2006;6:221. doi:10.1186/1471-2407-6-221, PMID:16959035.
- [98] Matsumoto S, Kikuchi A. Wnt/ β -catenin signaling pathway in liver biology and tumorigenesis. *In Vitro Cell Dev Biol Anim* 2024;60(5):466–481. doi:10.1007/s11626-024-00858-7, PMID:38379098.
- [99] So J, Martin BL, Kimelman D, Shin D. Wnt/ β -catenin signaling cell-autonomously converts non-hepatic endodermal cells to a liver fate. *Biol Open* 2013;2(1):30–36. doi:10.1242/bio.20122857, PMID:23336074.
- [100] Castelli G, Pelosi E, Testa U. Liver Cancer: Molecular Characterization, Clonal Evolution and Cancer Stem Cells. *Cancers (Basel)* 2017;9(9):E127. doi:10.3390/cancers9090127, PMID:28930164.
- [101] Kahn M. Wnt Signaling in Stem Cells and Cancer Stem Cells: A Tale of Two Coactivators. *Prog Mol Biol Transl Sci* 2018;153:209–244. doi:10.1016/b.pmbts.2017.11.007, PMID:29389517.
- [102] Ji J, Wang XW. Clinical implications of cancer stem cell biology in hepatocellular carcinoma. *Semin Oncol* 2012;39(4):461–472. doi:10.1053/j.seminoncol.2012.05.011, PMID:22846863.
- [103] Yan H, Li Z, Shen Q, Wang Q, Tian J, Jiang Q, *et al*. Aberrant expression of cell cycle and material metabolism related genes contributes to hepatocellular carcinoma occurrence. *Pathol Res Pract* 2017;213(4):316–321. doi:10.1016/j.prp.2017.01.019, PMID:28238542.
- [104] Karimaian A, Majidinia M, Bannazadeh Baghi H, Yousefi B. The cross-talk between Wnt/ β -catenin signaling pathway with DNA damage response and oxidative stress: Implications in cancer therapy. *DNA Repair (Amst)* 2017;51:14–19. doi:10.1016/j.dnarep.2017.01.003, PMID:28108274.
- [105] Jin Y, Anbarchian T, Wu P, Sarkar A, Fish M, Peng WC, *et al*. Wnt signaling regulates hepatocyte cell division by a transcriptional repressor cascade. *Proc Natl Acad Sci U S A* 2022;119(30):e2203849119. doi:10.1073/pnas.2203849119, PMID:35867815.
- [106] Xue W, Yang L, Chen C, Ashrafizadeh M, Tian Y, Sun R. Wnt/ β -catenin-driven EMT regulation in human cancers. *Cell Mol Life Sci* 2024;81(1):79. doi:10.1007/s00018-023-05099-7, PMID:38334836.
- [107] Mittal V. Epithelial Mesenchymal Transition in Tumor Metastasis. *Annu Rev Pathol* 2018;13:395–412. doi:10.1146/annurev-pathol-020117-043854, PMID:29414248.
- [108] Paolillo M, Schinelli S. Extracellular Matrix Alterations in Metastatic Processes. *Int J Mol Sci* 2019;20(19):4947. doi:10.3390/ijms20194947, PMID:31591367.
- [109] Wei Y, Van Nhieu JT, Prigent S, Srivatanakul P, Tiollais P, Buendia MA. Altered expression of E-cadherin in hepatocellular carcinoma: correlations with genetic alterations, beta-catenin expression, and clinical features. *Hepatology* 2002;36(3):692–701. doi:10.1053/jhep.2002.35342, PMID:12198663.
- [110] Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, *et al*. The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. *Cells* 2019;8(10):1118. doi:10.3390/cells8101118, PMID:31547193.
- [111] Zhai X, Zhu H, Wang W, Zhang S, Zhang Y, Mao G. Abnormal expression of EMT-related proteins, S100A4, vimentin and E-cadherin, is correlated with clinicopathological features and prognosis in HCC. *Med Oncol* 2014;31(6):970. doi:10.1007/s12032-014-0970-z, PMID:24781336.
- [112] Giannelli G, Koudelkova P, Ditturi F, Mikulits W. Role of epithelial to mesenchymal transition in hepatocellular carcinoma. *J Hepatol* 2016;65(4):798–808. doi:10.1016/j.jhep.2016.05.007, PMID:27212245.
- [113] Li LY, Yang JF, Rong F, Luo ZP, Hu S, Fang H, *et al*. ZEB1 serves an oncogenic role in the tumorigenesis of HCC by promoting cell proliferation, migration, and inhibiting apoptosis via Wnt/ β -catenin signaling pathway. *Acta Pharmacol Sin* 2021;42(10):1676–1689. doi:10.1038/s41401-020-00575-3, PMID:33514855.
- [114] Li W, Wu R, Zhang S, Zhao Y, Li L, Hu Z, *et al*. Analysis of angiogenesis-related subtypes of hepatocellular carcinoma and tumor microenvironment infiltration feature in hepatocellular carcinoma. *Clin Transl Oncol* 2023;25(7):2099–2115. doi:10.1007/s12094-023-03084-x, PMID:36708372.
- [115] Neth P, Ries C, Karow M, Egea V, Ilmer M, Jochum M. The Wnt signal transduction pathway in stem cells and cancer cells: influence on cellular invasion. *Stem Cell Rev* 2007;3(1):18–29. doi:10.1007/s12015-007-0001-y, PMID:17873378.
- [116] Astudillo P, Larraín J. Wnt signaling and cell-matrix adhesion. *Curr Mol Med* 2014;14(2):209–220. doi:10.2174/1566524014666140128105352, PMID:24467207.
- [117] Tejeda-Muñoz N, Morselli M, Moriyama Y, Sheladiya P, Pellegrini M, De Robertis EM. Canonical Wnt signaling induces focal adhesion and Integrin beta-1 endocytosis. *iScience* 2022;25(4):104123. doi:10.1016/j.isci.2022.104123, PMID:35402867.
- [118] Schambony A, Kunz M, Gradi D. Cross-regulation of Wnt signaling and cell adhesion. *Differentiation* 2004;72(7):307–318. doi:10.1111/j.1432-0436.2004.07207002.x, PMID:15554942.
- [119] Wang K, Qiu X, Zhao Y, Wang H, Chen L. The Wnt/ β -catenin signaling pathway in the tumor microenvironment of hepatocellular carcinoma. *Cancer Biol Med* 2021;19(3):305–318. doi:10.20892/j.issn.2095-3941.2021.0306, PMID:34591416.
- [120] Moenini A, Cornella H, Villanueva A. Emerging signaling pathways in hepatocellular carcinoma. *Liver Cancer* 2012;1(2):83–93. doi:10.1159/000342405, PMID:24159576.
- [121] Liu DX, Hao SL, Yang WX. Crosstalk Between β -CATENIN-Mediated Cell Adhesion and the WNT Signaling Pathway. *DNA Cell Biol* 2023;42(1):1–13. doi:10.1089/dna.2022.0424, PMID:36399409.
- [122] Zelikson N, Ben S, Caspi M, Tarabe R, Shaleve Y, Pri-Paz Basson Y, *et al*. Wnt signaling regulates chemokine production and cell migration of circulating human monocytes. *Cell Commun Signal* 2024;22(1):229. doi:10.1186/s12964-024-01608-8, PMID:38622714.
- [123] Qu B, Liu BR, DU YJ, Chen J, Cheng YQ, Xu W, *et al*. Wnt/ β -catenin signaling pathway may regulate the expression of angiogenic growth factors in hepatocellular carcinoma. *Oncol Lett* 2014;7(4):1175–1178. doi:10.3892/ol.2014.1828, PMID:24944688.
- [124] Yang C, Zhang H, Zhang L, Zhu AX, Bernards R, Qin W, *et al*. Evolving therapeutic landscape of advanced hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2023;20(4):203–222. doi:10.1038/s41575-022-00704-9, PMID:36369487.
- [125] Dahmani R, Just PA, Perret C. The Wnt/ β -catenin pathway as a therapeutic target in human hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol* 2011;35(11):709–713. doi:10.1016/j.clinre.2011.05.010, PMID:21778132.
- [126] Liu J, Pan S, Hsieh MH, Ng N, Sun F, Wang T, *et al*. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. *Proc Natl Acad Sci U S A* 2013;110(50):20224–20229. doi:10.1073/pnas.1314239110, PMID:24277854.
- [127] Gabata R, Harada K, Mizutani Y, Ouchi H, Yoshimura K, Sato Y, *et al*. Antitumor Activity of the Small Molecule Inhibitor PRI-724 Against β -Catenin-activated Hepatocellular Carcinoma. *Anticancer Res* 2020;40(9):5211–5219. doi:10.21873/anticancer.14524, PMID:32878809.
- [128] Huang Y, Sheng H, Xiao Y, Hu W, Zhang Z, Chen Y, *et al*. Wnt/ β -catenin inhibitor ICG-001 enhances the antitumor efficacy of radiotherapy by increasing radiation-induced DNA damage and improving tumor immune microenvironment in hepatocellular carcinoma. *Radiother Oncol* 2021;162:34–44. doi:10.1016/j.radonc.2021.06.034, PMID:34214613.
- [129] Gedaly R, Galuppo R, Daily MF, Shah M, Maynard E, Chen C, *et al*. Targeting the Wnt/ β -catenin signaling pathway in liver cancer stem cells and hepatocellular carcinoma cell lines with FH535. *PLoS One* 2014;9(6):e99272. doi:10.1371/journal.pone.0099272, PMID:24940873.
- [130] Ma L, Wang X, Jia T, Wei W, Chua MS, So S. Tankyrase inhibitors attenuate WNT/ β -catenin signaling and inhibit growth of hepatocellular carcinoma cells. *Oncotarget* 2015;6(28):25390–25401. doi:10.18632/oncotarget.4455, PMID:26246473.
- [131] Fischer MM, Cancilla B, Yeung VP, Cattaruzza F, Chartier C, Murriel CL, *et al*. WNT antagonists exhibit unique combinatorial antitumor activity with taxanes by potentiating mitotic cell death. *Sci Adv* 2017;3(6):e1700090. doi:10.1126/sciadv.1700090, PMID:28691093.
- [132] Chen L, Li M, Li Q, Wang CJ, Xie SQ. DKK1 promotes hepatocellular carcinoma cell migration and invasion through β -catenin/MMP7 signaling pathway. *Mol Cancer* 2013;12:157. doi:10.1186/1476-4598-12-157, PMID:24325363.
- [133] Zeyada MS, Abdel-Rahman N, El-Karef A, Yahia S, El-Sherbiny IM, Eissa LA. Niclosamide-loaded polymeric micelles ameliorate hepatocellular carcinoma in vivo through targeting Wnt and Notch pathways. *Life Sci* 2020;261:118458. doi:10.1016/j.lfs.2020.118458, PMID:32961231.
- [134] Wang F, He L, Dai WQ, Xu YP, Wu D, Lin CL, *et al*. Salinomycin inhibits proliferation and induces apoptosis of human hepatocellular carcinoma cells in vitro and in vivo. *PLoS One* 2012;7(12):e50638. doi:10.1371/journal.pone.0050638, PMID:23284640.
- [135] Harb J, Lin PJ, Hao J. Recent Development of Wnt Signaling Pathway Inhibitors for Cancer Therapeutics. *Curr Oncol Rep* 2019;21(2):12. doi:10.1007/s11912-019-0763-9, PMID:30715618.
- [136] Zhang X, Dong N, Hu X. Wnt/ β -catenin Signaling Inhibitors. *Curr Top Med Chem* 2023;23(10):880–896. doi:10.2174/1568026623666230303101810, PMID:36872364.
- [137] Emma MR, Augello G, Cusimano A, Azzolina A, Montalto G, McCubrey JA, *et al*. GSK-3 in liver diseases: Friend or foe? *Biochim Biophys Acta Mol Cell Res* 2020;1867(9):118743. doi:10.1016/j.bbamcr.2020.118743, PMID:32417256.
- [138] Katoh M. Multi-layered prevention and treatment of chronic inflammation, organ fibrosis and cancer associated with canonical WNT/ β -catenin signaling activation (Review). *Int J Mol Med* 2018;42(2):713–725. doi:10.3892/ijmm.2018.3689, PMID:29786110.
- [139] Lee SC, Kim OH, Lee SK, Kim SJ. IWR-1 inhibits epithelial-mesenchymal transition of colorectal cancer cells through suppressing Wnt/ β -catenin signaling as well as survivin expression. *Oncotarget* 2015;6(29):27146–27159. doi:10.18632/oncotarget.4354, PMID:26450645.
- [140] Tan Y, Lin XT, Luo YD, Zhang J, Fang L, Zhu YY, *et al*. Reduced I κ B α promotes hepatocellular carcinoma cell proliferation and migration via regulation of NF- κ B/Erbin axis. *Oncol Lett* 2020;20(5):216. doi:10.3892/ol.2020.12079, PMID:32963622.
- [141] Rothwarf DM, Karin M. The NF- κ B activation pathway: a paradigm in information transfer from membrane to nucleus. *Sci STKE* 1999;1999(5):RE1. doi:10.1126/stke.1999.5.re1, PMID:11865184.
- [142] Wu MY, Wang CC, Chang YC, Yu HY, Sung WW, Chen CJ, *et al*. The Therapeutic Role of PNU-74654 in Hepatocellular Carcinoma May Involve Suppression of NF- κ B Signaling. *Medicina (Kaunas)* 2022;58(6):798. doi:10.3390/medicina58060798, PMID:35744061.
- [143] Hwang SY, Deng X, Byun S, Lee C, Lee SJ, Suh H, *et al*. Direct Targeting of β -Catenin by a Small Molecule Stimulates Proteasomal Degradation and Suppresses Oncogenic Wnt/ β -Catenin Signaling. *Cell Rep* 2016;16(1):28–36. doi:10.1016/j.celrep.2016.05.071, PMID:27320923.
- [144] Pečina-Slaus N, Aničić S, Bukovac A, Kafka A. Wnt Signaling Inhibitors and Their Promising Role in Tumor Treatment. *Int J Mol Sci* 2023;24(7):6733. doi:10.3390/ijms24076733, PMID:37047705.
- [145] Lee HC, Kim M, Wands JR. Wnt/Frizzled signaling in hepatocellular carcinoma. *Front Biosci* 2006;11:1901–1915. doi:10.2741/1933, PMID:16368566.
- [146] Meng Z, Liu Q, Liu Y, Yang Y, Shao C, Zhang S. Frizzled-3 suppression

- overcomes multidrug chemoresistance by Wnt/ β -catenin signaling pathway inhibition in hepatocellular carcinoma cells. *J Chemother* 2023;35(7):653–661. doi:10.1080/1120009X.2023.2182573, PMID:36843499.
- [147] Koelman EMR, Yeste-Vázquez A, Grossmann TN. Targeting the interaction of β -catenin and TCF/LEF transcription factors to inhibit oncogenic Wnt signaling. *Bioorg Med Chem* 2022;70:116920. doi:10.1016/j.bmc.2022.116920, PMID:35841828.
- [148] Sheng J, Kohno S, Okada N, Okahashi N, Teranishi K, Matsuda F, *et al*. Treatment of Retinoblastoma 1-Intact Hepatocellular Carcinoma With Cyclin-Dependent Kinase 4/6 Inhibitor Combination Therapy. *Hepatology* 2021;74(4):1971–1993. doi:10.1002/hep.31872, PMID:33931882.
- [149] Dropmann A, Alex S, Schorn K, Tong C, Caccamo T, Godoy P, *et al*. The TGF- β 1 target WISP1 is highly expressed in liver cirrhosis and cirrhotic HCC microenvironment and involved in pro- and anti-tumorigenic effects. *Biochem Biophys Res Commun* 2024;732:150409. doi:10.1016/j.bbrc.2024.150409, PMID:39033550.
- [150] Naim A, Pan Q, Baig MS. Matrix Metalloproteinases (MMPs) in Liver Diseases. *J Clin Exp Hepatol* 2017;7(4):367–372. doi:10.1016/j.jceh.2017.09.004, PMID:29234202.
- [151] Scheau C, Badarau IA, Costache R, Caruntu C, Mihai GL, Didilescu AC, *et al*. The Role of Matrix Metalloproteinases in the Epithelial-Mesenchymal Transition of Hepatocellular Carcinoma. *Anal Cell Pathol (Amst)* 2019;2019:9423907. doi:10.1155/2019/9423907, PMID:31886121.
- [152] Wojtowicz-Praga SM, Dickson RB, Hawkins MJ. Matrix metalloproteinase inhibitors. *Invest New Drugs* 1997;15(1):61–75. doi:10.1023/a:1005722729132, PMID:9195290.
- [153] Xiao L, Wang M. Batimastat nanoparticles associated with transcatheter arterial chemoembolization decrease hepatocellular carcinoma recurrence. *Cell Biochem Biophys* 2014;70(1):269–272. doi:10.1007/s12013-014-9893-8, PMID:24639109.
- [154] Heath EI, O'Reilly S, Humphrey R, Sundaresan P, Donehower RC, Sartorius S, *et al*. Phase I trial of the matrix metalloproteinase inhibitor BAY12-9566 in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2001;48(4):269–274. doi:10.1007/s002800100330, PMID:11710626.
- [155] Lv Y, Zhao X, Zhu L, Li S, Xiao Q, He W, *et al*. Targeting intracellular MMPs efficiently inhibits tumor metastasis and angiogenesis. *Theranostics* 2018;8(10):2830–2845. doi:10.7150/thno.23209, PMID:29774078.
- [156] Belotti D, Paganoni P, Giavazzi R. MMP inhibitors: experimental and clinical studies. *Int J Biol Markers* 1999;14(4):232–238. doi:10.1177/172460089901400406, PMID:10669951.
- [157] Stevens AJ, Harris AR, Gerdtz J, Kim KH, Trentesaux C, Ramirez JT, *et al*. Programming multicellular assembly with synthetic cell adhesion molecules. *Nature* 2023;614(7946):144–152. doi:10.1038/s41586-022-05622-z, PMID:36509107.
- [158] Giannelli G, Bergamini C, Fransvea E, Marinosci F, Quaranta V, Antonaci S. Human hepatocellular carcinoma (HCC) cells require both α 5 β 1 integrin and matrix metalloproteinases activity for migration and invasion. *Lab Invest* 2001;81(4):613–627. doi:10.1038/labinvest.3780270, PMID:11304581.
- [159] Monvoisin A, Bisson C, Si-Tayeb K, Balabaud C, Desmoulière A, Rosenbaum J. Involvement of matrix metalloproteinase type-3 in hepatocyte growth factor-induced invasion of human hepatocellular carcinoma cells. *Int J Cancer* 2002;97(2):157–162. doi:10.1002/ijc.1595, PMID:11774258.
- [160] Erlichman C, Adjei AA, Alberts SR, Sloan JA, Goldberg RM, Pitot HC, *et al*. Phase I study of the matrix metalloproteinase inhibitor, BAY 12-9566. *Ann Oncol* 2001;12(3):389–395. doi:10.1023/a:1011183905848, PMID:11332153.
- [161] Giannelli G, Bergamini C, Marinosci F, Fransvea E, Quaranta M, Lupo L, *et al*. Clinical role of MMP-2/TIMP-2 imbalance in hepatocellular carcinoma. *Int J Cancer* 2002;97(4):425–431. doi:10.1002/ijc.1635, PMID:11802202.
- [162] Meng J, Sun B, Zhao X, Zhang D, Zhao X, Gu Q, *et al*. Doxycycline as an inhibitor of the epithelial-to-mesenchymal transition and vasculogenic mimicry in hepatocellular carcinoma. *Mol Cancer Ther* 2014;13(12):3107–3122. doi:10.1158/1535-7163.MCT-13-1060, PMID:25277383.
- [163] Lee KW, Kim MS, Kang NJ, Kim DH, Surh YJ, Lee HJ, *et al*. H-Ras selectively up-regulates MMP-9 and COX-2 through activation of ERK1/2 and NF-kappaB: an implication for invasive phenotype in rat liver epithelial cells. *Int J Cancer* 2006;119(8):1767–1775. doi:10.1002/ijc.22056, PMID:16721810.
- [164] Shalinsky DR, Brekken J, Zou H, McDermott CD, Forsyth P, Edwards D, *et al*. Broad antitumor and antiangiogenic activities of AG3340, a potent and selective MMP inhibitor undergoing advanced oncology clinical trials. *Ann N Y Acad Sci* 1999;878:236–270. doi:10.1111/j.1749-6632.1999.tb07689.x, PMID:10415735.
- [165] Sawada S, Murakami K, Yamaura T, Mitani N, Tsukada K, Saiki I. Therapeutic and analysis model of intrahepatic metastasis reflects clinical behavior of hepatocellular carcinoma. *Jpn J Cancer Res* 2002;93(2):190–197. doi:10.1111/j.1349-7006.2002.tb01258.x, PMID:11856483.
- [166] Liu FY, Wu YH, Zhou SJ, Deng YL, Zhang ZY, Zhang EL, *et al*. Minocycline and cisplatin exert synergistic growth suppression on hepatocellular carcinoma by inducing S phase arrest and apoptosis. *Oncol Rep* 2014;32(2):835–844. doi:10.3892/or.2014.3248, PMID:24919588.
- [167] Mita M, Kelly KR, Mita A, Ricart AD, Romero O, Tolcher A, *et al*. Phase I study of E7820, an oral inhibitor of integrin α -2 expression with antiangiogenic properties, in patients with advanced malignancies. *Clin Cancer Res* 2011;17(1):193–200. doi:10.1158/1078-0432.CCR-10-0010, PMID:21208908.
- [168] Ramakrishnan V, Bhaskar V, Law DA, Wong MH, DuBridge RB, Breinberg D, *et al*. Preclinical evaluation of an anti- α 5 β 1 integrin antibody as a novel anti-angiogenic agent. *J Exp Ther Oncol* 2006;5(4):273–286. PMID:17024968.
- [169] Delbaldo C, Raymond E, Vera K, Hammershaimb L, Kaucic K, Lozahic S, *et al*. Phase I and pharmacokinetic study of etaracizumab (Abegrin), a humanized monoclonal antibody against α 5 β 1 integrin receptor, in patients with advanced solid tumors. *Invest New Drugs* 2008;26(1):35–43. doi:10.1007/s10637-007-9077-0, PMID:17876527.
- [170] Cai J, Wang J, Jiang C, Ye L, He X, Huang J, *et al*. Combined inhibition of surface CD51 and γ -secretase-mediated CD51 cleavage improves therapeutic efficacy in experimental metastatic hepatocellular carcinoma. *J Hepatol* 2023;79(6):1418–1434. doi:10.1016/j.jhep.2023.08.007, PMID:37604269.
- [171] Chen W, Yang A, Jia J, Popov YV, Schuppan D, You H. Lysyl Oxidase (LOX) Family Members: Rationale and Their Potential as Therapeutic Targets for Liver Fibrosis. *Hepatology* 2020;72(2):729–741. doi:10.1002/hep.31236, PMID:32176358.
- [172] Goto K, Arai J, Stephanou A, Kato N. Novel therapeutic features of disulfiram against hepatocellular carcinoma cells with inhibitory effects on a disintegrin and metalloproteinase 10. *Oncotarget* 2018;9(27):18821–18831. doi:10.18632/oncotarget.24568, PMID:29721164.
- [173] Blaschuk OW. Potential Therapeutic Applications of N-Cadherin Antagonists and Agonists. *Front Cell Dev Biol* 2022;10:866200. doi:10.3389/fcell.2022.866200, PMID:35309924.
- [174] Kelley RK, Gane E, Assenat E, Siebler J, Galle PR, Merle P, *et al*. A Phase 2 Study of Galunisertib (TGF- β 1 Receptor Type I Inhibitor) and Sorafenib in Patients With Advanced Hepatocellular Carcinoma. *Clin Transl Gastroenterol* 2019;10(7):e00056. doi:10.14309/ctg.0000000000000056, PMID:31295152.
- [175] Wang K, Zhang K, Zhang X, Chen D, Jiang S. Recent Insights of Metformin on Hepatocellular Carcinoma (HCC). *Mini Rev Med Chem* 2023;23(11):1154–1166. doi:10.2174/1389557522666220623150717, PMID:35747976.
- [176] Fan X, Jin S, Li Y, Khadaroo PA, Dai Y, He L, *et al*. Genetic And Epigenetic Regulation Of E-Cadherin Signaling In Human Hepatocellular Carcinoma. *Cancer Manag Res* 2019;11:8947–8963. doi:10.2147/CMAR.S225606, PMID:31802937.
- [177] Xu F, Liu C, Zhou D, Zhang L. TGF- β /SMAD Pathway and Its Regulation in Hepatic Fibrosis. *J Histochem Cytochem* 2016;64(3):157–167. doi:10.1369/0022155415627681, PMID:26747705.
- [178] Ceballos MP, Parody JP, Alvarez Mde L, Ingaramo PI, Carnovale CE, Carrillo MC. Interferon- α 2b and transforming growth factor- β 1 treatments on HCC cell lines: Are Wnt/ β -catenin pathway and Smads signaling connected in hepatocellular carcinoma? *Biochem Pharmacol* 2011;82(11):1682–1691. doi:10.1016/j.bcp.2011.08.001, PMID:21843516.
- [179] Choucrair K, Kamran S, Saeed A. Clinical Evaluation of Ramucicrumab for the Treatment of Hepatocellular Carcinoma (HCC): Place in Therapy. *Oncotargets Ther* 2021;14:5521–5532. doi:10.2147/OTT.S268309, PMID:35002257.
- [180] Kuwano A, Yada M, Narutomi F, Nagasawa S, Tanaka K, Kurosaka K, *et al*. Therapeutic efficacy of atezolizumab plus bevacizumab for hepatocellular carcinoma with WNT/ β -catenin signal activation. *Oncol Lett* 2022;24(1):216. doi:10.3892/ol.2022.13337, PMID:35720502.
- [181] Huether A, Höpfner M, Baradari V, Schuppan D, Scherübl H. EGFR blockade by cetuximab alone or as combination therapy for growth control of hepatocellular cancer. *Biochem Pharmacol* 2005;70(11):1568–1578. doi:10.1016/j.bcp.2005.09.007, PMID:16226226.
- [182] Harkus U, Wankell M, Palamuthusingam P, McFarlane C, Hebbard L. Immune checkpoint inhibitors in HCC: Cellular, molecular and systemic data. *Semin Cancer Biol* 2022;86(Pt 3):799–815. doi:10.1016/j.semcancer.2022.01.005, PMID:35065242.
- [183] Donne R, Lujambio A. The liver cancer immune microenvironment: Therapeutic implications for hepatocellular carcinoma. *Hepatology* 2023;77(5):1773–1796. doi:10.1002/hep.32740, PMID:35989535.
- [184] Bupathi M, Kaseb A, Meric-Bernstam F, Naing A. Hepatocellular carcinoma: Where there is unmet need. *Mol Oncol* 2015;9(8):1501–1509. doi:10.1016/j.molonc.2015.06.005, PMID:26160430.
- [185] Thuru X, Magnez R, Vergoten G, Bailly C. A Potential Off-Target Effect of the Wnt/ β -Catenin Inhibitor KYA1797K: PD-L1 Binding and Checkpoint Inhibition. *Biomed Hub* 2023;8(1):1–9. doi:10.1159/000528499, PMID:36938364.
- [186] Zhong Z, Virshup DM. Wnt Signaling and Drug Resistance in Cancer. *Mol Pharmacol* 2020;97(2):72–89. doi:10.1124/mol.119.117978, PMID:31787618.
- [187] Tian M, Wang X, Sun J, Lin W, Chen L, Liu S, *et al*. IRF3 prevents colorectal tumorigenesis via inhibiting the nuclear translocation of β -catenin. *Nat Commun* 2020;11(1):5762. doi:10.1038/s41467-020-19627-7, PMID:33188184.
- [188] Katoh M. Canonical and non-canonical WNT signaling in cancer stem cells and their niches: Cellular heterogeneity, omics reprogramming, targeted therapy and tumor plasticity (Review). *Int J Oncol* 2017;51(5):1357–1369. doi:10.3892/ijo.2017.4129, PMID:29048660.