Mechanisms and Research Progress of the Wnt/β-catenin Signalling Pathway in Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is one of the most common primary liver cancers worldwide, and its development is a complex, multigenic, and multistep process. The Wnt/β-catenin signalling pathway, as a key regulator of cell proliferation, differentiation, apoptosis and migration, is frequently abnormally activated in HCC and represents a core molecular event promoting tumour initiation, progression, metastasis and treatment resistance.This article provides a systematic review of the core regulatory mechanisms of the Wnt/β-catenin signalling pathway, its role in the development of HCC, its interactions with the tumour microenvironment (TME) and other signalling pathways, as well as therapeutic strategies and research progress targeting this pathway. The aim is to provide a theoretical basis and new directions for the precision diagnosis and treatment of HCC.

***Keywords*:** Wnt/β-catenin signalling pathway; hepatocellular carcinoma; mechanism of action; tumour microenvironment; targeted therapy; research progress

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common and third leading cause of death from malignant tumours worldwide. It is highly heterogeneous, and its development is closely associated with viral hepatitis (HBV/HCV), alcoholic liver disease, and non-alcoholic fatty liver disease (NAFLD).Although surgical resection, liver transplantation, local ablation and systemic therapies (such as targeted drugs and immune checkpoint inhibitors) have significantly improved the prognosis for some patients, issues such as low early diagnosis rates, high postoperative recurrence rates and treatment resistance remain unresolved.

The Wnt/β-catenin signalling pathway is a highly evolutionarily conserved pathway that plays a key role in embryonic development, maintenance of liver homeostasis, and tissue regeneration. Abnormal activation of this pathway is closely associated with the development and progression of various tumours, and is particularly prominent in HCC.Approximately 20%–40% of HCC cases harbour gain-of-function mutations in CTNNB1 (which encodes β-catenin), whilst a further 3%–16% of cases exhibit loss-of-function mutations in AXIN1/2 or APC. These mutations lead to impaired β-catenin degradation and its accumulation within the cell nucleus, resulting in the sustained activation of downstream target gene transcription. Recent studies have shown that the Wnt/β-catenin pathway not only directly regulates the malignant phenotype of HCC cells but also participates in processes such as immune evasion and treatment resistance by reshaping the TME and acting in concert with other signalling pathways.This article integrates the latest research findings to comprehensively elucidate the mechanisms of action and research progress of the Wnt/β-catenin signalling pathway in HCC, providing new perspectives for targeted therapy of HCC [1].

2. Core Regulatory Mechanisms of the Wnt/β-catenin Signalling Pathway

2.1 Core Components and Regulation of the Classical Pathway

The Wnt/β-catenin signalling pathway is divided into the canonical and non-canonical pathways, with the canonical pathway being the most extensively studied in HCC [2].The canonical pathway primarily comprises Wnt ligands, Frizzled (FZD) receptors, LRP5/6 co-receptors, the cytoplasmic degradation complex (APC, GSK-3β, AXIN, CK1α), β-catenin, and nuclear TCF/LEF transcription factors.In the resting state, in the absence of Wnt ligands, cytoplasmic β-catenin binds to the degradation complex and is sequentially phosphorylated by CK1α (Ser45) and GSK-3β (Thr41, Ser37,Ser33), subsequently recognised and ubiquitinated by the E3 ubiquitin ligase β-TrCP, and finally degraded by the proteasome, thereby maintaining low levels of cytoplasmic β-catenin and keeping the transcription of downstream target genes suppressed.Upon binding of Wnt ligands to FZD receptors and LRP5/6 co-receptors, they recruit the cytoplasmic DVL protein, leading to the phosphorylation of LRP5/6 and the recruitment of the degradation complex, thereby inhibiting its phosphorylation of β-catenin.β-catenin

escapes degradation and accumulates in the cytoplasm, subsequently entering the cell nucleus where it binds to TCF/LEF transcription factors. It then recruits co-activators such as BCL9 and PYGO, thereby activating the transcription of downstream target genes including c-MYC, CCND1, MMP7 and AXIN2, and regulating biological processes such as cell proliferation, differentiation and migration.

2.2 Non-canonical regulatory mechanisms

In addition to classical regulation, the Wnt/β-catenin pathway is also finely regulated by various molecular mechanisms. Methylation of the promoters of Wnt pathway inhibitors, such as SFRP1, SFRP5 and DKK1, is a key mechanism for pathway activation in HCC. HBV/HCV infection can promote the nuclear translocation of β-catenin by inducing DNA methyltransferase activity, thereby suppressing the expression of SFRP family genes and lifting their inhibitory effect on the Wnt pathway.Long non-coding RNAs such as LncTCF7 and Lnc-β-Catm, as well as microRNAs such as miR-1246 and miR-5188, can influence pathway activity by regulating β-catenin stability, degradation complex assembly, or nuclear transcription activity.LncTCF7 can promote HCC cell proliferation by binding to miR-200c, thereby lifting its inhibition of β-catenin.

3. Mechanisms of action of the Wnt/β-catenin signalling pathway in the development of HCC

3.1 Promotion of HCC Cell Proliferation, Survival and Maintenance of Stem-like Properties

Abnormal activation of the Wnt/β-catenin pathway is one of the core drivers of malignant proliferation in HCC cells. Downstream target genes of the pathway, such as c-MYC and CCND1, can directly regulate the cell cycle, promoting the transition from the G1 phase to the S phase and accelerating cell proliferation.Enzymes such as MMP7 and MMP9 degrade the extracellular matrix, enhancing cell migration capacity and promoting tumour progression. Tumour stem cells are key to HCC recurrence and metastasis, and the Wnt/β-catenin pathway is a core signal for maintaining the stemness of tumour stem cells [3].

3.2 Mediation of HCC Invasion and Metastasis

Invasion and metastasis of HCC are the primary causes of patient mortality. The Wnt/β-catenin pathway promotes tumour progression by regulating epithelial-mesenchymal transition, cell migration and angiogenesis [4]. The nuclear accumulation of β-catenin activates epithelial-mesenchymal transition-related transcription factors such as SNAI1 and TWIST1, inhibits the expression of E-cadherin, and enhances the expression of N-cadherin and vimentin. This causes cells to lose epithelial polarity, acquire mesenchymal-like migratory capabilities, and promotes HCC cell invasion and distant metastasis.

3.3 Involvement in HCC Immune Evasion and Treatment Resistance

3.3.1 Mechanisms of Immune Evasion

Immune evasion in HCC is closely associated with the remodelling of the tumour microenvironment. The Wnt/β-catenin pathway shapes an immunosuppressive tumour microenvironment by regulating the infiltration and function of immune cells, thereby promoting immune evasion. This is achieved by upregulating the expression of molecules such as PD-L1 and CD24, which suppress T-cell activity and promote T-cell exhaustion.Wnt ligands can induce tumour-associated macrophages to polarise towards the M2 phenotype, secreting anti-inflammatory cytokines such as IL-10 and TGF-β, thereby suppressing anti-tumour immunity and promoting tumour progression. β-catenin can reduce the cytotoxic activity of natural killer cells against HCC cells by inhibiting the expression of NKG2D ligands such as ULBP1/2.Based on their immune status, HCC can be classified into immune-favored, immune-suppressed, and immune-intermediate types.

3.3.2 Mechanisms of Treatment Resistance

Abnormal activation of the Wnt/β-catenin pathway is a key factor in HCC resistance to targeted therapy and immunotherapy. Multi-targeted tyrosine kinase inhibitors such as sorafenib and lenvatinib are first-line treatments for HCC; β-catenin activation can upregulate the expression of multidrug resistance genes such as ABCB1 and ABCC1, promoting drug efflux.This pathway can also inhibit drug-induced apoptosis by activating anti-apoptotic pathways such as the PI3K/AKT pathway, thereby reducing treatment sensitivity [5].

4. Research Progress on Therapeutic Strategies Targeting the Wnt/β-catenin Signalling Pathway

Given the pivotal role of the Wnt/β-catenin pathway in HCC, therapeutic strategies targeting this pathway have become

a research focus, primarily encompassing small-molecule inhibitors, monoclonal antibodies, gene therapy and combination therapies [6].

4.1 Therapeutic Strategies Targeting the Cytoplasmic Degradation Complex

The core of strategies targeting the degradation complex lies in stabilising the complex or enhancing its ability to degrade β-catenin, primarily involving Tankyrase inhibitors and GSK-3β activators.

Tankyrase degrades the AXIN protein; its inhibitors, such as XAV939 and WXL-8, stabilise AXIN1/2, restore the function of the degradation complex, and inhibit the nuclear translocation of β-catenin, demonstrating significant antitumour activity in HCC cell lines.GSK-3β is the core kinase of the degradation complex; its activators, such as LY2090314, enhance β-catenin phosphorylation and degradation, inhibit the transcription of downstream target genes, and significantly suppress tumour growth in HCC animal models.

4.2 Therapeutic strategies targeting β-catenin nuclear translocation and transcriptional activity

Directly targeting β-catenin or its nuclear transcription complex represents the most direct therapeutic strategy, primarily involving β-catenin inhibitors and BCL9 inhibitors.PRI-724 specifically inhibits the interaction between β-catenin and BCL9, blocking the formation of the nuclear transcription complex, and has demonstrated antitumour activity in HCC cell lines and animal models [7]. Preclinical studies indicate that the combination of PRI-724 with sorafenib significantly enhances antitumour efficacy and overcomes drug resistance.BCL9 is a key co-activator of β-catenin nuclear transcription; its inhibitors, such as hsBCL9Z96, can inhibit the formation of the β-catenin/BCL9 complex, reprogram tumour-associated macrophages into the M1 phenotype, enhance antigen presentation, and restore T-cell antitumour activity. When used in combination with PD-L1 inhibitors, they can significantly reverse immunotherapy resistance [8].

4.3 Gene therapy strategies

Gene therapy achieves anti-tumour effects by regulating the expression of genes associated with the Wnt/β-catenin pathway [9], primarily including siRNA/miRNA therapy and CRISPR-Cas9 gene editing.Liposomal nanoparticles encapsulating CTNNB1 siRNA can specifically silence β-catenin expression; they have demonstrated significant antitumour activity in immunocompetent HCC models with β-catenin mutations, can activate type I/II interferon signalling, and reshape the immune microenvironment; their use in combination with immune checkpoint inhibitors can further enhance therapeutic efficacy.Knocking out key genes such as CTNNB1 and AXIN1 via the CRISPR-Cas9 system can block pathway activation and has demonstrated potential therapeutic value in HCC cell lines and animal models; however, further optimisation of the delivery system and safety assessment are required [10].

5. Conclusion

In summary, significant progress has been made in elucidating the mechanisms of action of the Wnt/β-catenin signalling pathway in HCC; however, key challenges such as specificity, drug resistance and delivery still need to be overcome. In the future, through the synergistic advancement of precision targeting, multi-pathway combination therapies, the development of novel technologies and clinical translation research, it is hoped that more effective and safer treatment strategies can be provided for HCC patients, thereby further improving patient prognosis.

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