Dysregulation of Wnt/β-catenin signaling by protein kinases in hepatocellular carcinoma and its therapeutic application

Qian Li¹ | Mengqing Sun¹ | Menglan Wang¹ | Mengqing Feng¹ | Fan Yang¹ | Lina Li¹ | Jianbo Zhao¹ | Cunjie Chang¹ | Heng Dong¹ | Tian Xie¹ | Jianxiang Chen¹,²

¹Key Laboratory of Elemene Class Anti-Cancer Chinese Medicines, Engineering Laboratory of Development and Application of Traditional Chinese Medicines, Collaborative Innovation Center of Traditional Chinese Medicines of Zhejiang Province, Department of Hepatology, Institute of Hepatology and Metabolic Diseases, Institute of Integrated Chinese and Western Medicine for Oncology, The Affiliated Hospital of Hangzhou Normal University, College of Pharmacy, School of Medicine, Hangzhou Normal University, Hangzhou, China
²Division of Cellular and Molecular Research, Laboratory of Cancer Genomics, National Cancer Centre, Singapore City, Singapore

Correspondence
Tian Xie and Jianxiang Chen, Department of Hepatology, Institute of Hepatology and Metabolic Diseases, Institute of Integrated Chinese and Western Medicine for Oncology, The Affiliated Hospital of Hangzhou Normal University, Key Laboratory of Elemene Class Anti-Cancer Chinese Medicines, Engineering Laboratory of Development and Application of Traditional Chinese Medicines, Collaborative Innovation Center of Traditional Chinese Medicines of Zhejiang Province, College of Pharmacy, School of Medicine, Hangzhou Normal University, Hangzhou, Zhejiang 311121, China.
Emails: xbs@hznu.edu.cn (T.X.); chenjx@hznu.edu.cn (J.C.)

Abstract
Wnt/β-catenin signaling is indispensable for many biological processes, including embryonic development, cell cycle, inflammation, and carcinogenesis. Aberrant activation of the Wnt/β-catenin signaling can promote tumorigenicity and enhance metastatic potential in hepatocellular carcinoma (HCC). Targeting this pathway is a new opportunity for precise medicine for HCC. However, inhibiting Wnt/β-catenin signaling alone is unlikely to significantly improve HCC patient outcome due to the lack of specific inhibitors and the complexity of this pathway. Combination with other therapies will be an important next step in improving the efficacy of Wnt/β-catenin signaling inhibitors. Protein kinases play a key and evolutionarily conserved role in the Wnt/β-catenin signaling and have become one of the most important drug targets in cancer. Targeting Wnt/β-catenin signaling and its regulatory kinase together will be a promising HCC management strategy. In this review, we summarize the kinases that modulate the Wnt/β-catenin signaling in HCC and briefly discuss their molecular mechanisms. Furthermore, we list some small molecules that target the kinases and may inhibit Wnt/β-catenin signaling, to offer new perspectives for preclinical and clinical HCC studies.

Keywords
β-catenin signaling, combination therapy, hepatocellular carcinoma, protein kinase, small molecule, Wnt

Abbreviations: AMPK, AMP-activated protein kinase; APC, adenomatous polyposis coli; CDK1, cyclin-dependent kinase 1; CK1, casein kinase 1; Dvl, Dishevelled; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; FGFR, fibroblast growth factor receptor; GSK3β, glycogen synthase kinase 3β; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; JNK, Jun N-terminal kinase; LEP/TCF, lymphoid enhancer factor/T cell factor; LRPS/6, low density lipoprotein receptor-related protein 5/6; MAPK, mitogen-activated protein kinase; Met, mesenchymal-epithelial transition factor; NEK2, NIMA-related kinase 2; NF-κB, nuclear factor-κB; PAK4, 21st activated kinase 4; PI3K, phosphatidylinositol 3-kinase; ROR2, tyrosine kinase-like orphan receptor 2; RTK, receptor tyrosine kinase; SK1, salt-inducible kinase 1; STAT, signal-transducer and activator of transcription; TAK1, transforming growth factor-β activated kinase 1; TGF-β, transforming growth factor-β; VEGFRs, vascular endothelial growth factor receptors; WCS, Wnt/β-catenin signaling.

Qian Li and Mengqing Sun contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2021 The Authors. Cancer Science published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association.
1 | INTRODUCTION

Liver cancer is the sixth most common cancer and the fourth leading cause of cancer mortality worldwide, with approximately 841,000 new cases and 782,000 deaths annually. Hepatocellular carcinoma (HCC) is the most common type and accounts for 75%-85% of all liver cancer cases, the main risk factors for HCC are hepatitis B or C virus infection, alcohol abuse, and aflatoxin-contaminated foodstuffs. The clinical management of HCC mainly includes surgical therapies, tumor ablation, transarterial therapies, and systemic therapies. Although these management strategies have developed quickly over the past decade, the prognosis is still dismal, with a 5-y survival rate of 18%. Therefore, a better understanding of the molecular mechanisms involved in HCC initiation and progression is crucial for the identification of therapeutic targets and the design of specific drugs. Excessive activation of the Wnt/β-catenin signaling (WCS) in hepatocytes leads to uncontrollable growth, expansion, and metastasis of malignant clones, indicating a great potential for WCS as a drug target in HCC.

There are approximately 518 protein kinase genes in the human genome, targeting one-third of the proteins in cells. Protein kinases modulate most of the signal transduction pathways in humans, as well as most cellular processes, including metabolism, transcription, translation, cell cycle, and proliferation. Mutations, overexpression, and dysfunction of protein kinases play essential roles in the pathogenesis of cancer, and kinase has become one of the most important drug targets over the past 20 y. To date, the US Food and Drug Administration (FDA) has approved 38 kinase inhibitors, most of which are RTK inhibitors for the clinical treatment of cancer. Protein kinases have been reported to regulate the WCS in the pathogenesis of HCC and could be targeted specifically. Therefore, in this review we summarize the kinases reported that modulate the WCS in HCC; the small molecules that targeting these kinases could be applied for HCC clinical treatment.

2 | THE OVERVIEW OF WCS

The Wnt signaling pathways are mechanically complex and relatively conserved pathways associated with many physiological and pathological processes such as embryonic development, tissue self-renewal, and cancer. Currently, there are 3 summarized pathways upon Wnt stimulation: the WCS, the Wnt/Ca²⁺ pathway and the non-canonical planar cell polarity (PCP) pathway. Of these 3, the WCS is most well studied. The hallmark of this pathway is that it activates the transcriptional activity of β-catenin, which is the key mediator of WCS. β-Catenin in the cytoplasm is tightly regulated by the destruction complex, formed by scaffold protein Axin, APC, casein kinase 1 (CK1) and glycogen synthase kinase 3β (GSK3β). In the absence of Wnt ligands, the destruction complex captures and phosphorylates β-catenin, which is subsequently recognized by the E3 ubiquitin ligase β-TRCP and targeted for proteasomal degradation. When the Wnt ligand binds the heterodimeric receptor complex formed by Fizzled (Fz) and low density lipoprotein receptor-related protein5/6 (LRP5/6) at the plasma membrane, the signal is primed by LRP5/6 phosphorylation, activating and recruiting Dishevelled (Dvl) to form an aggregation platform and the LRP6 signalosome, composed of Wnt, Fz, Axin, phosphorylated LRP5/6, GSK3β and CK1, which eventually disrupt the destruction complex and promote stabilization and cytoplasmic accumulation of β-catenin. Free β-catenin can translocate into the nucleus, and bind lymphoid enhancer factor/T cell factor (LEF/TCF) transcription factor complex to promote the transcription of Wnt target genes, including Myc, cyclin D1 and Axin2 (Figure 1).

The WCS is inactive in the normal condition of liver, however it is frequently mutated and activated in HCC. There are multiple regulatory ways in which hepatocarcinogenesis is sustained by the WCS. For instance, the protein regulator of cytokinesis 1 (PRC1)/Wnt positive feedback loop plays a crucial role in HCC recurrence and metastasis. Targeting WCS is a new opportunity for precise medicine for HCC. To date, many clinical agents, such as small molecules, peptides and antibodies, have been developed to modulate the WCS in HCC. These agents target Wnt ligands, inhibit the Fz-Dvl interaction, or stabilize the β-catenin destruction complex. However, inhibiting WCS alone is unlikely to significantly improve HCC patient outcome due to the lack of specific inhibitors and the complexity of this pathway. Combination with other therapies will be an important next step in improving the utility of WCS inhibitors. Protein kinase has become one of the most important drug targets in cancer, targeting WCS and its regulatory kinase together would be the preferred choice. A deeper understanding of the WCS and its regulatory kinase would provide opportunities to design better combination therapies.

3 | KINASES MODULATING WCS

The dysfunction of kinases results in significant changes in most cellular signal transduction processes, including in the WCS. In HCC,
many protein kinases have been reported to modulate the WCS at various levels (Table 1 and Figure 2). Some protein kinases directly involved in WCS regulation target and phosphorylate the key components in WCS, modulating β-catenin degradation, LRP6 signaling, and LEF/TCF/β-catenin transcription complex activity. Other kinases indirectly modulate the WCS, and this modulation varies from kinase to kinase.

4 | GSK3β

GSK3β is an evolutionarily conserved serine/threonine kinase that is ubiquitously expressed in mammalian cells and functions in diverse cellular processes including proliferation, differentiation, and motility. GSK3β is directly involved in the WCS, acting as a component of the β-catenin destruction complex to promote cytoplasmic β-catenin degradation by sequential phosphorylation in cooperation with CK1. In addition, GSK3β phosphorylates Axin1 and increases its binding to β-catenin, allowing the N-terminal region of β-catenin to become a more efficient substrate of CK1 and GSK3β. GSK3β also phosphorylates APC and increases its affinity for β-catenin, followed by ubiquitination of phosphorylated β-catenin by β-TRCP. In addition to its inhibitory role, GSK3β positively regulates the WCS by phosphorylation of LRP6. Wnt induces sequential phosphorylation of LRP6 by GSK3β and CK1, and this dual phosphorylation recruits Axin1 away from the β-catenin destruction complex to the LRP6 signalosome. Recently, it has been
| Kinase name | Expression in TCGA HCC compared with normal liver tissue | Mechanisms | Positive/negative modulation of Wnt signal | Reference |
|------------|-------------------------------------------------|-------------|------------------------------------------|------------|
| GSK3β     | Up                                             | 1. GSK3β phosphorylates APC, Axin1 and β-catenin to facilitate the ubiquitination and proteasomal degradation of β-catenin.  
2. GSK3β phosphorylates LRP6 and induces the formation of LRP6 signalosome.  
3. GSK3β potentially inhibits many Wnt signaling-associated factors by promoting their protein degradation by phosphorylation. | Both | 13-20 |
| CK1       | Up                                             | 1. CK1 phosphorylates APC and β-catenin to facilitate the ubiquitination and proteasomal degradation of β-catenin.  
2. CK1 phosphorylates LRP6 and induces the formation of LRP6 signalosome.  
3. CK1 phosphorylates TCF3 and promotes its interaction with β-catenin.  
4. CK1 phosphorylates Dvl and promotes the signaling activity of Dvl. In addition, the phosphorylation of Dvl by CK1 also triggers a negative feedback loop to inhibit the Wnt/β-catenin signaling.  
5. CK1 phosphorylates p120-catenin and cadherin, resulting in release of β-catenin from the junctional complex. | Both | 24-30 |
| CK2       | Up                                             | 1. CK2 phosphorylates β-catenin, leading to its proteasome resistance.  
2. The phosphorylation of LEF-1 by CK2 significantly enhances its affinity for β-catenin and stimulates the transactivation of β-catenin:LEF-1 complexes. | Positive | 34,35 |
| CDK1      | Up                                             | CDK1 could directly phosphorylate BCL9, which would stabilize BCL9, inhibit clathrin binding to the BCL9/LRP6 complex and suppress clathrin-mediated degradation of LRP6 signalosome | Positive | 40 |
| CDK14     | Up                                             | CDK14/cyclin Y phosphorylates LRP5/6 and activates mitotic Wnt/β-catenin signaling | Positive | 39 |
| Met       | Up                                             | Met phosphorylates β-catenin at tyrosine residues, this causes dissociation of β-catenin from Met and nuclear translocation of β-catenin | Positive | 44 |
| EGFR      | Down                                           | 1. EGFR directly phosphorylates β-catenin, which leads to release of β-catenin from junctional complexes and increase of cytoplasmic β-catenin concentration.  
2. EGFR activated ERK phosphorylates LRP6 and dramatically increases the cellular response to Wnt ligand. | Positive | 48 |
| NEK2      | Up                                             | 1. NEK2 could bind to β-catenin to prevent its ubiquitination and degradation.  
2. Dvl accumulated at centrosome is phosphorylated by NEK2, this phosphorylation releases Dvl from the centrosome and increases the Wnt/β-catenin signaling activation. | Positive | 51,54 |
| PAK4      | Up                                             | PAK4 phosphorylates β-catenin and prevents its degradation from proteasome pathway | Positive | 56 |
| Src       | Up                                             | 1. Src phosphorylates Fz, allowing Fyn recruitment and activation. Fyn phosphorylates β-catenin, releasing β-catenin from the junctional complexes.  
2. Src phosphorylates β-catenin, resulting in the accumulation of β-catenin in the nucleus and the promotion of LEF/TCF transcription.  
3. The phosphorylation of LRP6 by Src reduces LRP6 levels on the cell surface, disrupts LRP6 signalosome formation. | Both | 60-62 |
| TAK1      | No significant difference                      | The activation of TAK1 promotes NLK activity, NLK could phosphorylate TCF and interfere with the binding of β-catenin-TCF to the TCF target sites | Negative | 66 |
| SIK1      | Down                                           | SIK1 phosphorylates SMRT at threonine 1391 and promotes its translocation into the nucleus, then phosphorylated SMRT recruits the NCoR/HDAC3 corepressor complex to β-catenin/TCF/LEF and inhibits the transcription of Wnt target genes | Negative | 71 |

(Continues)
indicated that the Wnt ligand triggers the sequestration of GSK3β from the cytosol into multivesicular bodies (MVBs) to promote cytoplasmic β-catenin accumulation and, ultimately, activation of the WCS. Moreover, GSK3β potentially inhibits many Wnt signaling-associated proteins by promoting their degradation by phosphorylation. However, the WCS stabilizes the GSK3β targeting proteins, especially in G2/M, indicating that this Wnt-dependent stabilization of proteins may be an alternative mode of the WCS, especially in mitosis.

**TABLE 1** (Continued)

| Kinase name | Expression in TCGA HCC compared with normal liver tissue | Mechanisms | Positive/negative modulation of Wnt signal | Reference |
|-------------|--------------------------------------------------------|------------|------------------------------------------|-----------|
| FAK         | Up                                                     | FAK reduces β-catenin degradation and increases the nuclear accumulation of β-catenin | Positive   | 75         |
| ROR2        | Up                                                     | ROR2 directly binds Wnt5a, inhibits the Wnt/β-catenin signaling and activates non-canonical Wnt signaling | Negative   | 76,77      |

Abbreviations: APC, adenomatous polyposis coli; CDK1, cyclin-dependent kinase 1; CK1, casein kinase 1; Dvl, Dishevelled; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; Fz, Frizzled; GSK3β, glycogen synthase kinase 3β; HCC, hepatocellular carcinoma; HDAC3, histone deacetylase 3; LEF/TCF, lymphoid enhancer factor/T cell factor; LRP5/6, low density lipoprotein receptor-related protein 5/6; Met, mesenchymal-epithelial transition factor; NCoR, nuclear receptor corepressor; NLK, NEMO-like kinase; PAK4, P21 activated kinase 4; ROR2, tyrosine kinase-like orphan receptor 2; SIK1, salt-inducible kinase 1; SMRT, silencing mediators of retinoic acid and thyroid hormone receptor; TAK1, transforming growth factor-β (TGF-β) activated kinase 1; TCF3, transcription factor 3.

**FIGURE 2** Kinases that modulate the Wnt/β-catenin signaling. Various protein kinases have been reported to modulate the Wnt/β-catenin signaling in HCC, with some directly phosphorylating the components of this pathway. These protein kinases generally regulate through 3 mechanisms. First, they target β-catenin, Axin1, APC, and so on to regulate the stability, accumulation, and location of β-catenin, such as GSK3β, CK1, CK2, NEK2, FAK, PAK4, Met, and EGFR. Second, they phosphorylate LRP6 and affect the formation of the LRP6 signalosome, such as CK1 and Src. Finally, they target β-catenin and LEF/TCF and modulate the LEF/TCF/β-catenin transcription complex, such as CK1 and CK2. In addition, other kinases indirectly modulate the Wnt/β-catenin signaling, and this modulation varies from kinase to kinase, such as SIK1. The small-molecule inhibitors and activators of the kinases which may be used in combination therapy are indicated in this figure in blue boxes. Abbreviations: APC, adenomatous polyposis coli; CK1, casein kinase 1; CK2, casein kinase 2; Dvl, Dishevelled; EGFR, epidermal growth factor receptor; FAK, Focal adhesion kinase; GSK3β, glycogen synthase kinase 3β; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; LEF/TCF, lymphoid enhancer factor/T cell factor; LRP6, low density lipoprotein receptor-related protein 6; Met, mesenchymal-epithelial transition factor; NEK2, NIMA-related kinase 2; PAK4, P21 activated kinase 4; SIK1, Salt-inducible kinase 1.
GSK3β in HCC remain controversial,21 it positively and negatively modulates the WCS. Therefore, whether these modulations contribute to the occurrence and development of HCC remains unclear.

5 | CK1 AND CK2

CK1 and CK2 are serine/threonine kinases, ubiquitously expressed from yeast to humans. Mammalian CK1 possesses 7 family members: α, β, γ1, γ 2, γ 3, δ, and ε, while CK2 is a tetramer consisting of 2 catalytic subunits: CK2α and CK2α', and 2 regulatory subunits: CK2β and CK2β'.22,23 CK1 regulates diverse cellular processes, such as circadian rhythms, Wnt signaling, membrane trafficking, and cytoskeleton maintenance.23 CK2 is involved in key cellular processes, such as inhibition of apoptosis, DNA damage response, and cell cycle regulation.22

Almost all members of the CK1 family have been indicated in WCS regulation, with particular roles. Interestingly, CK1 isoforms have been identified as both positive and negative regulators of the WCS. Similar to GSK3β, CK1 activates the WCS through the phosphorylation of LRP6 (mostly by CK1α, CK1ε/δ and CK1γ in this case)24,25 and inhibits it through the phosphorylation of β-catenin (mainly by CK1ε,26 and APC (by CK1ε and CK1δ).27 Transcription Factor 3 (TF3) phosphorylation by CK1ε promotes its interaction with β-catenin and activates the WCS.28 Dvl phosphorylation by CK1ε has been well studied, this phosphorylation promotes Dvl signaling and, therefore, the WCS.29 In addition to this activating function, CK1ε triggers a negative feedback loop to inhibit the WCS. Dvl has the capability to multimerize and cluster Wnt-receptor complexes into LRP6 signalosomes. The phosphorylation of Dvl via CK1ε promotes the Huwe1-dependent ubiquitination of Dvl, thereby inhibiting Dvl multimerization and, therefore, inhibiting the WCS.30 Low cytoplasmic CK1ε expression is correlated with a low survival rate and a high probability of tumor vascular invasion in HCC patients, indicating that CK1ε may function as a tumor suppressor in HCC.31

β-Catenin is a multifunctional protein that can associate with the transcription complex and junctional complex at the cell membrane where β-catenin interacts with proteins such as α-catenin, p120-catenin and cadherin to mediate cell-cell adhesion.32 The Wnt-induced sequestration of GSK3β into MVBs is regulated by the junctional complex and CK1α. In Wnt-stimulated cells, the junctional complex binds the LRP6 signalosome, and CK1α phosphorylates p120-catenin and cadherin, resulting in the separation of the 2 complexes and the release of β-catenin from the junctional complex, finally activating the WCS. Separated LRP6 signalosome, including GSK3β, are internalized to MVBs.33

CK2 appears to be capable of affecting the WCS at multiple levels. CK2 phosphorylates β-catenin at threonine 393, leading to proteasome resistance, and ultimately potentiating the WCS.34 Furthermore, the phosphorylation of nuclear LEF1 via CK2 significantly enhances its affinity for β-catenin and stimulates trans-activation of the β-catenin/LEF1 complex.35 CK2α is aberrantly overexpressed in HCC,36 and knockdown of CK2α results in significant apoptosis and inhibition of the migration and invasion of HCC cells,37 confirming that CK2α could be a marker of poor prognosis for HCC. Based on these findings, it can be concluded that overexpressed CK2α positively modulates the WCS and promotes HCC progression.

6 | CYCLIN-DEPENDENT KINASE 1 (CDK1) AND CDK14

CDK1 and CDK14 are serine/threonine kinases with reported roles in the regulation of the cell cycle by phosphorylating protein substrates-associated with specific cyclin subunits.38 Mitotic WCS has been discovered to be constitutively activated during mitosis due to phosphorylation of LRP5/6 by CDK14/cyclin Y, and the phosphorylation site has been reported to be the same as that used by GSK3β.39 Recently, we found that CDK1 could directly and markedly phosphorylate mitotic BCL9 on its N-terminal, especially at threonine 172. This can stabilize BCL9 and inhibit binding of the clathrin complex to the BCL9/LRP6 signalosome to suppress both clathrin-mediated endocytosis and the subsequent degradation of the LRP6 signalosome, and also modulate the WCS dynamically and precisely during mitosis.40 This CDK1/BCL9/mitotic Wnt signaling pathway is dynamically controlled by CDK1 activity during mitosis, undergoing higher activity in prophase and metaphase, and a relatively decreased activity in anaphase and telophase. These processes confirmed that CDK1 is directly involved in regulating the WCS, at least by phosphorylating BCL9 in mitosis. More Wnt-related substrates of CDK1 could be revealed in the future to further link CDKs with the WCS in biological and physiological stages or disease processes. CDK1 and CDK14 are upregulated in HCC in accordance with TCGA data, indicating that their positive modulations in WCS may contribute to HCC progression.

7 | MET

RTKs are a subclass of tyrosine kinases that have emerged as key regulators of a wide range of complex biological functions, including cell growth, motility, differentiation, and metabolism.41 In humans, there are 58 known RTKs, and these have been categorized into 20 subfamilies including EGFR, mesenchymal-epithelial transition factor (Met), fibroblast growth factor receptors (FGFR), and ROR. The Met RTK family contains 3 members: Met, Ron, and c-Sea. Met is the receptor for HGF, and the HGF/Met axis modulates the multiple intracellular signaling pathways involved in muscle and liver formation, cell proliferation, morphogenesis and motility, and epithelial-mesenchymal transition.42 Tumorigenic Met mutants (M1268T) contribute to the cellular accumulation of β-catenin and constitutive activation of the WCS, which is partially due to β-catenin tyrosine phosphorylation by mutated Met.43 Further investigation has revealed that Met is associated with β-catenin at the cell membrane
and, upon HGF stimulation, activated Met phosphorylates β-catenin at tyrosine residues. This causes dissociation of β-catenin from Met and the nuclear translocation of β-catenin, resulting in the expression of Wnt target genes.\textsuperscript{44} Aberrant activation of Met promotes the initiation, proliferation, invasion, and metastasis of HCC. HCC patients with a high expression of Met have significantly lower survival rates than the patients with a low or no expression of Met.\textsuperscript{45} Based on these findings, it can be concluded that upregulated Met positively modulates the WCS and promotes HCC progression. Moreover, Met plays a critical role in drug resistance, therefore targeting the HGF/Met axis has been one of the most promising therapies for HCC.\textsuperscript{56}

8 | EGFR AND FGFR

Upon ligand binding, activated EGFR and FGFRs turn on downstream signaling pathways such as the Ras-Raf-MEK-ERK1/2 pathway, STAT pathways, and the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, to control cell proliferation, survival, and differentiation.\textsuperscript{47} Intriguingly, FGFR2, FGFR3, and EGFR also significantly activate the WCS through 2 different mechanisms. First, FGFR2, FGFR3, and EGFR directly phosphorylate β-catenin at tyrosine 142, which leads to the release of β-catenin from membrane junctions and an increase of cytoplasmic β-catenin. Second, ERK phosphorylates LRP6 at serine 1490 and threonine 1572 during its Golgi network-based maturation process, and this phosphorylation dramatically promotes the cellular response to the Wnt ligand.\textsuperscript{48} Notably, EGFR is frequently overexpressed in human HCC, and its upregulation is correlated with aggressive tumors, metastasis, and poor patient survival.\textsuperscript{47} According to this research, EGFR may act as an oncogene, partially by enhancing the WCS in HCC. However, EGFR inhibitors have achieved only modest results in HCC clinical trials.\textsuperscript{49}

9 | NIMA-RELATED KINASE 2 (NEK2)

NEK2 is a chromosomal instability associated gene that encodes a serine/threonine kinase and belongs to the never in mitosis A (NIMA)-related family of kinases. NEK2 plays a key role in regulating mitotic processes, including centrosome duplication and separation, microtubule stability, kinetochore and microtubule attachment and mitotic spindle formation.\textsuperscript{50} In G2/M phase, Dvl accumulates at the centrosomes and is phosphorylated by NEK2 at several residues, this increases its affinity toward linker proteins at the centrosomes. Dvl then acts as a scaffold to form a complex with the linker proteins and facilitates the release of the complex from the centrosomes. The Dvl released from the centrosome is available for, and increases, WCS activation.\textsuperscript{51} Several studies have confirmed that NEK2 is aberrantly overexpressed in human HCC tissues and cell lines, and its expression is significantly correlated with the progression of HCC patients.\textsuperscript{52,53} Moreover, NEK2 induces sorafenib resistance, a first-line treatment for advanced-stage HCC, via WCS activation. In sorafenib-treated HCC cell lines, NEK2 binds and stabilizes β-catenin, promoting its translocation to the nucleus, which consequently activates the transcription of Wnt target genes.\textsuperscript{54} Therefore, NEK2 acts as a potential therapeutic target to improve the response of HCC patient to sorafenib treatment, especially those resistant to this agent.

10 | P21 ACTIVATED KINASE 4 (PAK4)

PAK4 is a serine/threonine kinase that was originally identified as a downstream effector of the small Rho GTPases, such as Rac1 and Cdc42. It plays vital roles in many biological functions such as cell growth, cell survival, cytoskeletal organization, cell migration, and morphology.\textsuperscript{55} PAK4 has known links to the WCS, and it can shuttle between the cytoplasm and nucleus to positively modulate this pathway. In the cytoplasm, PAK4 phosphorylates β-catenin and prevents its degradation from proteasome pathway. Nuclear accumulation of PAK4 enhances the nuclear import of β-catenin, promotes Wnt target gene transcription and upregulates β-catenin expression.\textsuperscript{56} Interestingly, the methylation of PAK4 has also been connected to the WCS. SETD6, a protein lysine methyltransferase, binds and methylates PAK4 on chromatin, which enhances the interaction between PAK4 and β-catenin, promotes the formation of an active β-catenin/TCF complex, and results in the activation of the WCs.\textsuperscript{57} Importantly, PAK4 is aberrantly upregulated in HCC and contributes to cancer metastasis,\textsuperscript{58} indicating that it may contribute to HCC progression by enhancing the WCS.

11 | SRC TYROSINE KINASES

Src family kinases are nonreceptor tyrosine kinases that act downstream of RTKs and integrins to regulate cell proliferation, adhesion, morphology, and movement.\textsuperscript{59} Src family kinases include isoforms such as Src, Blk (B-lymphoid tyrosine kinase), Fyn, and Lck (Lymphocyte specific kinase). Src is activated by phosphorylation at the key residue tyrosine 416 and dephosphorylation at tyrosine 527. Src acts as either a positive or negative regulator of the WCS. Upon Wnt3a stimulation, Src binds to, and is activated by Dvl, and then Src phosphorylates Dvl and β-catenin, resulting in the accumulation of β-catenin in the nucleus, promoting LEF/TCF-mediated transcription of Wnt target genes.\textsuperscript{50} Src is also associated with and phosphorylates LRP6 directly, which reduces LRP6 expression levels on the cell surface, disrupts LRP6 signalosome formation, and negatively regulates the WCS.\textsuperscript{61} Intriguingly, Src and Fyn modulate a new signaling cascade that is different from the WCS. Upon Wnt3a stimulation, activated Src phosphorylates Fz, allowing Fyn recruitment and activation. Fyn phosphorylates β-catenin, releasing β-catenin from the junctional complex to stimulate Wnt target gene expression.\textsuperscript{62} Notably, the protein expression of Src and tyrosine 416 phosphorylated Src (p-Y416Src) are significantly higher in HCC tissues compared with adjacent normal tissues.\textsuperscript{63,64} Increased expression of p-Y416Src is associated with poor patient survival, suggesting that p-Y416Src may serve as an independent prognostic marker for patient survival.
in HCC. Although Src is an oncogene in HCC, it has dual functions in regulating the WCS. Further investigations are needed to clarify whether these regulations contribute to HCC progression.

12 | TRANSFORMING GROWTH FACTOR-β (TGF-β) ACTIVATED KINASE 1 (TAK1)

TAK1 is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) superfamily, it is activated by TGF-β and regulates both nuclear factor-κB (NF-κB) and MAPKs signaling pathways, which play key roles in embryogenesis, development, inflammation, the immune response, and metabolism.65 TAK1 has been reported to be involved in the WCS by stimulating NEMO-like kinase (NLK) activity and inhibiting the transcriptional activation. TAK1 activation promotes the activity of NLK, which can phosphorylate TCF and then interfere with the binding of β-catenin-TCF to the TCF binding element, thereby negatively regulating the WCS.66 More recent studies have revealed that Wnt1 can directly activate the TAK1-NLK cascade, resulting in the phosphorylation of TCF.67 Therefore, Wnt signal transduction through the WCS activates β-catenin/TCF whereas, through the TAK1-NLK pathway, it phosphorylates and inhibits TCF, which might function as a feedback mechanism. Hepatocyte-specific deletion of TAK1 in mice results in spontaneous hepatocyte dysplasia, liver inflammation, and the development of HCC, indicating that it acts as a tumor suppressor.68 TAK1 may exert anti-tumor effects by inhibiting the WCs in HCC.

13 | SALT-INDUCIBLE KINASE 1 (SIK1)

SIK1 is a serine/threonine protein kinase belonging to the AMP-activated protein kinase (AMPK) family. SIK1 is activated by liver kinase B1 phosphorylation and plays crucial roles in a series of cellular processes, including cell proliferation and apoptosis.59 The expression of SIK1 is significantly downregulated in HCC, and the regulation of SIK1 occurs at both the transcriptional and post-transcriptional levels.70 Recently, it has been reported that the loss of SIK1 accelerates HCC growth and invasion through activation of WCS. Mechanistically, SIK1 phosphorylates the silencing mediators of retinoic acid and thyroid hormone receptor (SMRT) at threonine 1391 and promotes its translocation into the nucleus. Then, phosphorylated SMRT recruits the nuclear receptor corepressor (NCoR)/histone deacetylase 3 (HDAC3) corepressor complex to β-catenin/TCF and inhibits the transcription of Wnt target genes. Loss of SIK1 leads to the dephosphorylation of SMRT and its export from the nucleus, therefore activating the WCS.71

14 | FOCAL ADHESION KINASE (FAK)

FAK is a highly conserved non-RTK encoded by the protein tyrosine kinase 2 gene in humans. FAK acts at the intersection of various signaling pathways, including PI3K/Akt signaling and JNK signaling.72 When activated, FAK controls cell adhesion, proliferation, migration, and cancer stem cell self-renewal through both kinase-dependent and kinase-independent mechanisms.72 FAK protein and mRNA are overexpressed in HCC compared with corresponding normal liver tissues and are positively correlated with tumor stage, vascular invasion, and intrahepatic metastasis.73,74 Recently, FAK has been reported to stimulate the WCS by inhibiting β-catenin degradation and promoting its nuclear accumulation in HCC. In this manner, FAK promotes a cancer stem cell-like phenotype and enhances tumorigenicity, leading to HCC recurrence and sorafenib resistance.75 However, the precise molecular mechanism by which FAK regulates β-catenin in HCC remains unclear.

15 | TYROSINE KINASE-LIKE ORPHAN RECEPTOR 2 (ROR2)

ROR2 is a member of the ROR RTK family, with functional extracellular Wnt-binding domains and is implicated in Wnt signal transduction. ROR2 was first found to bind directly to the non-canonical Wnt ligand Wnt5a, to inhibit the WCS, with no effect on the regulation of cellular calcium and β-catenin levels.76 In addition, ROR2 functions as a co-receptor of Wnt5a and activates non-canonical Wnt signaling. After Wnt5a binds to Fz, it interacts with ROR2 and recruits GSK3β to phosphorylate ROR2 at serine 864, which is required for the activation of ROR2 function. Interestingly, Wnt5a and the canonical Wnt ligand Wnt3a compete for binding to Fz at the cell surface. The identity of the Wnt ligand can determine whether the Fz co-receptor would be LRP5/6 or ROR2, therefore dictating whether Wnt/β-catenin-dependent or -independent signaling would be activated, respectively.77 However, the details of how activated ROR2 modulates Wnt signaling is completely unknown, and elucidation of more downstream components of ROR2 would provide a more detailed mechanisms of Wnt/ROR2 signal transduction. TCGA data revealed that ROR2 is upregulated in HCC, however, it negatively modulates the WCS. Therefore, it may not promote HCC progression by enhancing the WCS.

16 | TARGETING THE KINASES RELATED WITH WCS IN HCC

To date, increasing numbers of studies on the molecular mechanisms of tumorigenesis have shown that protein kinases play pivotal roles in various types of cancer. Because of mutation or overexpression, some constitutively active protein kinases promote cancer cell proliferation and survival, therefore they are considered oncogenic.78 Other kinases expressed in the tumor or surrounding tissues are also required for tumor maintenance such as the VEGFRs, which are important for inducing angiogenesis.79 Surprisingly, treating cells with kinase inhibitors does not cause excessive damage to normal cells, therefore these inhibitors can be used to selectively kill tumor cells.80 For the above reasons, kinases have become important targets in
TABLE 2 Kinase inhibitors or activators modulating Wnt/β-catenin signaling potentially in HCC

| Target | Molecule | Product description | Reference |
|--------|----------|---------------------|-----------|
| GSK3β | Pyrvinium pamoate | It is an FDA-approved anti-parasite drug, it activates GSK3β to phosphorylate β-catenin for degradation and ultimately inhibits Wnt/β-catenin signaling | 85 |
| CDK1  | Roninibiclib | This drug targets CDK1 with IC_{50} of 7 nM. It has entered phase II clinical trials for the treatment of solid tumor | 86 |
|        | Dinaciclib | This drug targets CDK1 with IC_{50} of 3 nM. It has entered phase III clinical trials for the treatment of chronic lymphocytic leukemia | 87 |
| CK2   | CX-4945 | This drug targets CK2 with IC_{50} of 1.5 nM. It has entered phase II clinical trials for the treatment of multiple myeloma, brain, and gastrointestinal cancer | 88 |
| FAK   | PF-562271 | This drug targets FAK with IC_{50} of 1.5 nM. Its phase I clinical trials for the treatment of advanced solid cancers has completed | 89 |
|        | Defactinib | This drug targets FAK. It has entered phase II clinical trials for the treatment of advanced solid cancers | 90 |
| Src   | Saracatinib | This drug targets Src, Lyn, Fyn, Blk, EGFR with IC_{50} of 2.7, 5, 10, 11, 66 nM, respectively. Its phase II clinical trials for the treatment of advanced solid cancers has completed | 91 |
| Met   | Cabozantinib | This drug targets Met and VEGFR2 with IC_{50} of 1.3 and 0.035 nM, respectively. It is in phase IV clinical trials for the treatment of HCC | 92 |
|        | Tepotinib | This drug targets Met with IC_{50} of 4 nM. It is in phase II clinical trials for the treatment of HCC | 93 |
|        | Golvatinib | This drug targets Met and VEGFR2 with IC_{50} of 14 and 16 nM, respectively. It is in phase II clinical trials for the treatment of advanced solid tumors | 94 |
|        | Capmatinib | This drug targets Met with IC_{50} of 0.13 nM. It is in phase II clinical trials for the treatment of advanced HCC | 95 |

Abbreviations: Blk, B-lymphoid tyrosine kinase; CDK1, cyclin-dependent kinase 1; CK2, casein kinase 2; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; GSK3β, glycogen synthase kinase 3β; HCC, hepatocellular carcinoma; Met, mesenchymal-epithelial transition factor; VEGFR2, vascular endothelial growth factor receptor 2.

(Continues)

cancer treatment. For instance, sorafenib and lenvatinib are small-molecule multikinase inhibitors, both of which are US FDA approved for first-line systemic treatment of advanced-stage HCC patients and have been shown to prolong survival.80 Met is emerging as a biologically rational target in HCC and it positively modulates the WCS. Met inhibitor crizotinib has been reported to inhibit the WCS.81 A phase I study of Met inhibitors combined with sorafenib has illustrated the safety of the combination strategy,80,82 therefore the combination of Met inhibitors with WCS inhibitors may synergistically enhance their anti-tumor effect and may be a safe and promising treatment approach. Pyrvinium pamoate is an FDA-approved anti-parasite drug. A previous study revealed that it selectively activates CK1α to suppress the WCS,83 however a further study supported that it did not activate CK1α, but alternatively activated GSK3β to phosphorylate β-catenin for degradation and ultimately inhibited the WCS.84,85 Pyrvinium pamoate dose dependently inhibited cancer stem cell regeneration and proliferation in various breast cancer cell lines, indicating that it is a feasible agent for HCC therapy. Some small molecules targeting the kinases mentioned above are selectively listed (Table 2) to offer new perspectives for preclinical and clinical HCC studies.

17  |  CONCLUSION AND PERSPECTIVE

The initiation and development of HCC is a complex process with many factors and stages.97 The WCS is frequently hyperactivated in HCC, and a substantial proportion of these patients with HCC have β-catenin mutations. Targeting the WCS represents a new opportunity for HCC treatment that is currently under clinical investigation.83 Protein phosphorylation modulates almost every aspect of cellular processes, including the WCS, and protein kinase dysfunction is the cause of many diseases, especially cancer. Consequently, protein kinases are emerging as the second largest group of drug targets after G protein coupled receptors.4 In this review, we focus on protein kinases that modulated the WCS in HCC. CDK1, CDK14, CK2, NEK2, FAK, PAK4, Met, and EGFR activate the WCS, while TAK1, SIK1 and ROR2 inhibit it, and GSK3β, CK1, and Src have dual functions. Most of these kinases phosphorylate WCS components and directly regulate this pathway through different mechanisms.
Other kinases target non-WCS components, indirectly regulating the pathway in various ways. Some regulation of WCS by protein kinases has been linked to HCC, for example downregulated SIK1 phosphorylates SMRT, promotes its translocation into the nucleus and recruits the NCoR/HDAC3 corepressor complex to $\beta$-catenin/TCF/LEF, inhibiting the transcription of Wnt target genes and ultimately contributing to HCC.71 However, the relationship between some other regulations and HCC remains unclear, for example GSK3β regulation, and needs to be explored in the future. Given the abundance of kinase substrates and the pivotal role of the WCS in HCC, an increasing number of kinases will be discovered to modulate this pathway directly or indirectly in prospective studies.

With the advent of personalized precision medicine, it is now clear that each HCC patient is unique and that the mechanisms of HCC occurrence and progression may be diverse in different patients.92 Therefore, single target inhibitors aimed at one molecule or pathway are not universally effective and have the problem of drug resistance resulting from target gene mutations or upregulation of alternative signaling pathways.46 Strategies for combinations of multiple drugs targeting multiple targets are urgently needed.4 Thus far, targeting the WCS and its regulatory kinases will provide a theoretical basis for better targeting of cancer. Furthering our understanding of the crosstalk between the WCS and its regulatory kinases will be possible and is an promising research direction to be explored in the near future.98,99

ACKNOWLEDGMENTS

This work was supported by grants from the Start-up Grant of HZNU (412SCS021820470), National Natural Science Foundation of China (81802338, 82072646) (to JC); National Natural Science Foundation of China (81973635, 81730108) (to TX); National Natural Science Foundation of China (81802831) (to CC); National Natural Science Foundation of China (81902507) (to QL); Zhejiang Provincial Natural Science Foundation of China for Distinguished Young Scholars (LR21H160001) (to JC); Zhejiang Provincial Natural Science Foundation of China (LY21H160043) (to CC); Zhejiang Provincial Natural Science Foundation of China (LY21H160044) (to QL).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Qian Li https://orcid.org/0000-0002-2560-5730

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.

2. Villanueva A. Hepatocellular carcinoma. N Engl J Med. 2019;380:1450-1462.

3. Perugorria MJ, Olaizola P, Labiano I, et al. Wnt-beta-catenin signaling in liver development, health and disease. Nat Rev Gastroenterol Hepatol. 2019;16:121-136.

4. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer. 2009;9:28-39.

5. Ferguson FM, Gray NS. Kinase inhibitors: the road ahead. Nat Rev Drug Discov. 2018;17:353-377.

6. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2000;127:469-480.

7. Nusse R, Clevers H. Wnt/beta-catenin signaling, disease, and emerging therapeutic modalities. Cell. 2017;169:985-999.

8. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol. 2004;20:781-810.

9. Wang W, Smits R, Hao H, He C. Wnt/beta-catenin signaling in liver cancers. Cancers. 2019;11(7):926.

10. Chen J, Rajasekaran M, Xia H, et al. The microtubule-associated protein PRC1 promotes early recurrence of hepatocellular carcinoma in association with the Wnt/beta-catenin signalling pathway. Gut. 2016;65:1522-1534.

11. Anastas JN, Moon RT. Wnt signalling pathways as therapeutic targets in cancer. Nat Rev Cancer. 2013;13:11-26.

12. Luo J. Glycogen synthase kinase 3beta (GSK3beta) in tumorigenesis and cancer chemotherapy. Cancer Lett. 2009;273:194-200.

13. Tejeda-Munoz N, Robles-Flores M. Glycogen synthase kinase 3 in Wnt signaling pathway and cancer. JUMBS Life. 2015;67:914-922.

14. Jho E-H, Lomvardas S, Costantini F. A GSK3β phosphorylation site in axin modulates interaction with β-catenin and Tcf-mediated gene expression. Biochem Biophys Res Comm. 1999;266:28-35.

15. Dajani R, Fraser E, Roe SM, et al. Structural basis for recruitment of glycogen synthase kinase 3beta to the axin-APC scaffold complex. The EMBO Journal. 2003;22:494-501.

16. Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P. Binding of GSK3β to the APC-β-catenin complex and regulation of complex assembly. Science. 1996;272:1023.

17. Su Y, Fu C, Ishikawa S, et al. APC is essential for targeting phosphorylated β-catenin to the SCFβ-TrCP ubiquitin ligase. Mol Cell. 2008;32:652-661.

18. Bilic J, Huang YL, Davidson G, et al. Wnt induces LRP6 signalosomes and recruits the NCoR/HDAC3 corepressor complex to β-catenin/TCF/LEF, inhibiting the transcription of Wnt target genes and ultimately contributing to HCC.71

19. Taelman VF, Dobrowolski R, Plouhinec JL, et al. Wnt signaling promotes protein stabilization and regulates cell size. Mol Cell. 2017;54:663-674.

20. Taelman VF, Dobrowolski R, Plouhinec JL, et al. Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. Cell. 2010;143:1136-1148.

21. Acerbon SP, Karaulanov E, Berger BS, Huang YL, Niehrs C. Mitotic wnt signaling promotes protein stabilization and regulates cell size. Mol Cell. 2014;54:663-674.

22. Chua MM, Ortega CE, Sheikh A, et al. CK2 in cancer: cellular and biochemical mechanisms and potential therapeutic target. Pharmaceuticals. 2017;10(4):18.

23. Jiang J, CK1 in developmental signaling: hedgehog and Wnt. Curr Top Dev Biol. 2017;123:303-329.

24. Davidson G, Wu W, Shen J, et al. Casein kinase 1 gamma couples Wnt receptor activation to cytoplasmic signal transduction. Nature. 2005;438:867-872.

25. Zeng X, Tamai K, Doble B, et al. A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. Nature. 2005;438:873-877.

26. Liu C, Li Y, Fau-Semenov M, et al. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell. 2002;108:837-847.

27. Ha N-C, Tonozuka T, Stamos JL, Choi H-J, Weis WI. Mechanism of phosphorylation-dependent binding of APC to β-catenin and its role in β-catenin degradation. Mol Cell. 2004;15:511-521.

28. Lee E, Salic A, Kirschner MW. Physiological regulation of [beta]-catenin stability by Tcf3 and CK1epsilon. J Cell Biol. 2001;154:983-993.
29. Klomowsky LK, Garcia BA, Shabanowitz J, Hunt DF, Virshup DM. Site-specific casein kinase 1 epsilon-dependent phosphorylation of Dishevelled modulates beta-catenin signaling. The FEBS Journal. 2006;273:4594-4602.

30. de Groot RE, Ganji RS, Bernatik O, et al. Huwe1-mediated ubiquitilation of dishevelled defines a negative feedback loop in the Wnt signaling pathway. Sci Signal. 2014;7:ra26.

31. Lin SH, Yeh CM, Hsieh MJ, et al. Low cytoplasmic casein kinase 1 epsilon expression predicts poor prognosis in patients with hepatocellular carcinoma. Tumour Biol. 2016;37:3997-4005.

32. Harris TJ, Peifer M. Decisions, decisions: beta-catenin chooses between adhesion and transcription. Trends Cell Biol. 2005;15:234-237.

33. Vinyoles M, Del Valle-Perez B, Curto J, et al. Multivesicular GSK3 sequestration upon Wnt signaling is controlled by p120-catenin/cadherin interaction with LRP5/6. Mol Cell. 2014;53:444-457.

34. Song DH, Dominguez I, Mizuno J, Kaut M, Mohr SC, Seldin DC. CK2 phosphorylation of the armadillo repeat region of beta-catenin potentiates Wnt signaling. The Journal of Biological Chemistry. 2003;278:24018-24025.

35. Wang S, Jones KA. CK2 controls the recruitment of Wnt regulators to target genes in vivo. Curr Biol. 2006;16:2239-2244.

36. Zhang HX, Jiang SS, Zhang XF, et al. Protein kinase CK2alpha catalytic subunit is overexpressed and serves as an unfavorable prognostic marker in primary hepatocellular carcinoma. Oncotarget. 2015;6:34800-34817.

37. Wu D, Su C, Meng F, et al. Stable knockdown of protein kinase CK2alpha (CK2alpha) inhibits migration and invasion and induces inactivation of hedgehog signaling pathway in hepatocellular carcinoma Hep G2 cells. Acta Histochem. 2014;116:1501-1508.

38. Malumbres M, Barbacid M. Mammalian cyclin-dependent kinases. Trends Biochem Sci. 2005;30:630-641.

39. Davidson G, Shen J, Huang YL, et al. Cell cycle control of wnt receptor activation. Dev Cell. 2009;17:788-799.

40. Chen J, Rajasekaran M, Xia H, et al. CDK1-mediated BCL9 phosphorylation inhibits clathrin to promote mitotic Wnt signalling. The EMBO Journal. 2018;37.

41. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010;141:1117-1134.

42. Wang H, Rao B, Lou J, et al. The function of the HGF/c-met axis in hepatocellular carcinoma. Front Cell Dev Biol. 2020;8:55.

43. Danilkovitch-Miagkova A, Miagkov A, Skeel A, Nakaigawa N, Zbar B, Leonard EJ. Oncogenic mutants of RON and MET receptor tyrosine kinases cause activation of the beta-catenin pathway. The Journal of Biological Chemistry. 2016;291:6786-6795.

44. Xu HT, Lai WL, Liu HF, Wong LL, Ng IO, Ching YP. PKA phosphorylates p53 at serine 215 to promote liver cancer metastasis. Can Res. 2016;76:5732-5742.

45. Patel A, Sabineni H, Clarke A, Somanath PR. Novel roles of Src in cancer cell epithelial-to-mesenchymal transition, vascular permeability, microinvasion and metastasis. Life Sci. 2016;157:52-61.

46. Yokoyama N, Malbon CC. Dishevelled-2 docks and activates Src in a Wnt-dependent manner. J Cell Sci. 2009;122:4439-4451.

47. Chen Q, Su Y, Wessolowski J, et al. Tyrosine phosphorylation of LRP6 by Src and Fer inhibits Wnt/beta-catenin signalling. EMBO Rep. 2014;15:1254-1267.

48. Villarroel A, Del Valle-Perez B, Fuertes G, et al. Src and Fyn define a new signaling cascade activated by canonical and non-canonical Wnt ligands and required for gene transcription and cell invasion. Cell Mol Life Sci 2020;77(5):919-935.

49. Zhao R, Wu Y, Wang T, et al. Elevated Src expression associated with hepatocellular carcinoma metastasis in northern Chinese patients. Oncol Lett. 2015;10:3026-3034.

50. Roh YS, Song J, Seki E. TAK1 regulates hepatic cell survival and carcinogenesis. J Gastroenterol. 2014;49:185-194.

51. Ishitan T, Ninomiya-Tsuji J, Nagai S, et al. The TAK1-NLK-MAPK-related pathway antagonizes signalling between beta-catenin and transcription factor TCF. Nature. 1999;399:798-802.

52. Smit L, Baas A, Kuipers J, Korswagen H, van de Wetering M, Clevers H. Wnt activates the Tak1/Nemo-like kinase pathway. The Journal of Biological Chemistry. 2004;279:17232-17240.

53. Bettmann K, Vucur M, Haybaeck J, et al. TAK1 suppresses a NEMO-dependent but NF-kB-independent pathway to liver cancer. Cancer Cell. 2010;17:481-496.

54. Wein MN, Foretz M, Fisher DE, Xavier RJ, Kronenberg HM. Salt-inducible kinases: physiology, regulation by cAMP, and therapeutic potential. Trends Endocrinol Metab. 2018;29:723-735.

55. Qu C, Qu YQ. Down-regulation of salt-inducible kinase 1 (SIK1) is mediated by RNF2 in hepatocarcinogenesis. Oncotarget. 2017;8:3144-3155.

56. HCC progression and WNT/beta-catenin activation. J Hepatol. 2016;64:1076-1089.
87. Parry D, Guzi T, Shanahan F, et al. Dinaciclib (SCH 727965), a novel cyclin-dependent kinase inhibitor. Mol Cancer Ther. 2010;9:2344-2353.

88. Siddiqui-Jain A, Drygin D, Streiner N, et al. CX-4945, an orally bioavailable selective inhibitor of protein kinase CK2, inhibits prosurvival and angiogenic signaling and exhibits antitumor efficacy. Can Res. 2010;70:10288-10298.

89. Roberts WG, Ung E, Whalen P, et al. Antitumor activity and pharmacology of a selective focal adhesion kinase inhibitor, PF-562,271. Can Res. 2008;68:1935-1944.

90. Jones SF, Siu LL, Bendell JC, et al. A phase I study of VS-6063, a second-generation focal adhesion kinase inhibitor, in patients with advanced solid tumors. Invest New Drugs. 2015;33:1100-1107.

91. Green TP, Fennell M, Whittaker R, et al. Preclinical anticancer activity of the potent, oral Src inhibitor AZD0530. Mol Oncol. 2009;3:248-261.

92. Yakes FM, Chen J, Tan J, et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. Mol Cancer Ther. 2011;10:2298-2308.

93. Bladt F, Faden B, Friese-Hammin M, et al. EMD 1214063 and EMD 1204831 constitute a new class of potent and highly selective c-Met inhibitors. Clin Cancer Res. 2013;19:2941-2951.

94. Nakagawa T, Toyohama O, Yamaguchi A, et al. E7050: a dual c-Met and VEGFR-2 tyrosine kinase inhibitor promotes tumor regression and prolongs survival in mouse xenograft models. Cancer Sci. 2010;101:210-215.

95. Liu X, Wang Q, Yang G, et al. A novel kinase inhibitor, INCB28060, blocks c-MET-dependent signaling, neoplastic activities, and cross-talk with EGFR and HER-3. Clin Cancer Res. 2011;17:7127-7138.

96. Moyer JD, Barbacci EG, Iwata KK, et al. Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Can Res. 1997;57:4838-4848.

97. Craig AJ, von Felden J, Garcia-Lezana T, Sarcognato S, Villanueva A. Tumour evolution in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2020;17:139-152.

98. Etnyre D, Stone AL, Fong JT, et al. Targeting c-Met in melanoma: mechanism of resistance and efficacy of novel combinatorial inhibitor therapy. Cancer Biol Ther. 2014;15:1129-1141.

99. Ellerkamp V, Lieber J, Nagel C, et al. Pharmacological inhibition of beta-catenin in hepatoblastoma cells. Pediatr Surg Int. 2013;29:141-149.