Review

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Wnt signaling and hepatocarcinogenesis: Molecular targets for the development of innovative anticancer drugs

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Summary

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer death worldwide. HCC can be cured by radical therapies if early diagnosis is done while the tumor has remained of small size. Unfortunately, diagnosis is commonly late when the tumor has grown and spread. Thus, palliative approaches are usually applied such as transarterial intrahepatic chemoembolization and sorafenib, an anti-angiogenic agent and MAP kinase inhibitor. This latter is the only targeted therapy that has shown significant, although moderate, efficiency in some individuals with advanced HCC. This highlights the need to develop other targeted therapies, and to this goal, to identify more and more pathways as potential targets. The Wnt pathway is a key component of a physiological process involved in embryonic development and tissue homeostasis. Activation of this pathway occurs when a Wnt ligand binds to a Frizzled (FZD) receptor at the cell membrane. Two different Wnt signaling cascades have been identified, called non-canonical and canonical pathways, the latter involving the β-catenin protein. Deregulation of the Wnt pathway is an early event in hepatocarcinogenesis and has been associated with an aggressive HCC phenotype, since it is implicated both in cell survival, proliferation, migration and invasion. Thus, component proteins identified in this pathway are potential candidates of pharmacological intervention. This review focuses on the characteristics and functions of the molecular targets of the Wnt signaling cascade and how they may be manipulated to achieve anti-tumor effects.

Keywords: Hepatocellular carcinoma; Therapy; Wnt pathway; Drug discovery.
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Abbreviations: HCC, hepatocellular carcinoma; FZD, frizzled; CSC, cancer stem cells; PCP, planar cell polarity; TCF/LEF, T-cell factor/lymphoid enhancer factor; TLE-1, transducin like enhancer-1; APC, adenomatous polyposis coli protein; GSK3β, glycogen synthase kinase 3β; CK1, casein kinase 1; β-TRCP, beta-transducin repeat containing protein; SCF, Skp1/cullin F-box complex; Dvl/Dsh, dishevelled; Pygo, pygopus; CBP, CREB-binding protein; PKC, protein kinase C; NFAT, nuclear factor of activated T cell; NLK, Nemo-like kinase; Wif1, Wnt inhibitory protein-1; sFRP, secreted FZD-related proteins; Dkk, Dickkopf; Krm, Kremen; Rspo, R-spondin; Gpc3, glypican-3; DIFs, differentiation-inducing factors.

Introduction

HCC represents a major public health problem with a high impact on society. HCC is the sixth most common tumor worldwide in terms of incidence (about one million *per* year). Projections are that this incidence will substantially increase during the next decades due to persistent infection with the hepatitis C virus as well as the emergence of non-alcoholic steatohepatitis as a major health problem. HCC portends a poor prognosis since ranking third in terms of "cause of death" by cancer, and often presents as a major complication of cirrhosis related to chronic hepatitis B and C infections, or non-virus related [1–3]. The dismal prognosis is generally related to a late diagnosis after HCC cells have infiltrated the liver parenchyma, have spread through the portal venous system and/or have formed distant metastases. However, if HCC is diagnosed early (<20% of patients), these smaller tumors may be cured by surgical resection, liver transplantation or radiofrequency ablation. In more advanced tumors (>80% of patients at diagnosis), only palliative approaches can be applied. In this regard, transarterial intrahepatic chemoembolization has been shown to be somewhat effective in increasing overall survival of individuals with tumors that have spread only into the liver parenchyma without extrahepatic metastasis (median overall survival is increased from 15 to 20 months compared to the best supportive care). In HCC with extrahepatic spread, only sorafenib, an anti-angiogenic and MAP kinase inhibitor, has been shown to increase overall survival of patients (from 8 to 11 months) [4]. All other systemic approaches such as cytotoxic chemotherapy have not been shown to be effective; thus, to date, no targeted therapy except sorafenib has been proven to prolong life in patients with HCC. However, there are ongoing or ended clinical trials with agents that target FGF, VEGF, PDGF, EGF, IGF, mTOR, and TGFβ signaling pathways but none has been shown yet to have a significant impact on patient survival [5].

Recently, cancer stem cells (CSC) have been hypothesized to play a key role in tumor maintenance as well as relapse after surgical resection. There is accumulating information that supports a role for CSC in hepatocarcinogenesis to maintain the tumor size and to initiate tumor recurrence following therapy [6]. The pool of CSC is maintained by self-renewal capabilities that are largely driven by reactivation of embryonic signaling programs mediated by Wnt, Notch, Bmi, and Hedgehog pathways, similar to what has been previously demonstrated during breast carcinogenesis [7]. Preclinical studies further underline the potential value of inhibiting activation of these signaling programs in some tumor types [8–11].

In this review, we describe the features of a therapeutic target, i.e., the Wnt pathway, for potential therapy of HCC. We will discuss experimental and preclinical studies regarding the use of Wnt inhibitors as a therapeutic approach for HCC.

The Wnt-mediated signaling

The first member of the Wnt family of ligands was identified from the *int-1* gene found in a mammary adenocarcinoma, located at the integration site of the mouse mammary tumor virus (MMTV); subsequently, it was demonstrated to have oncogenic properties [12]. More important, *int-1* homolog genes have been found in human tumors as well [13]. In addition, a highly conserved *int-1* homolog was also discovered in Drosophila and designated *Wingless* "Wg" [14]. The combination of *int-1* and *Wingless* led to the common Wnt1 terminology and recently has been used to designate the Wnt family of ligands [15].

Wnt proteins are secreted extracellular auto-paracrine glycoproteins that interact with Frizzled receptors (FZD), a seven transmembrane domain protein, resembling the G-protein-coupled receptor (GPCR) family. Vinson and colleagues revealed that FZD contains an extracellular cysteine-rich domain (CRD) which is the putative binding site for the Wnt ligands. These investigators demonstrated the functional role of the *frizzled* locus to coordinate development of the cytoskeleton in Drosophila epidermal cells [16]. Subsequently, Wnt/FZD-mediated signaling has been extensively studied, and although it has been widely implicated in cellular homeostasis, these ligand/receptor interactions have now been appreciated as key factors during the oncogenesis process and therefore, could serve as new therapeutic targets.

Thus, Wnt proteins represent members of a highly conserved family that is involved in several processes including embryonic development, cell fate determination, proliferation, polarity, migration, and stem cell maintenance. In addition, Wnt/beta-catenin signaling has been found to play key roles in metabolic zonation of adult liver, regeneration [17]. In adult organisms, deregulation of Wnt signaling may lead to tumor development [18,19]. The Wnt-mediated pathway is activated through the binding of one Wnt ligand to a FZD receptor. Ten different FZD receptors and 19 Wnt ligands have been identified in humans. The binding of Wnt to an FZD receptor can trigger activation of at least three different pathways. The first is the Wnt/β-catenin cascade, also called the Wnt-canonical pathway; the remaining two are the planar cell polarity (PCP) and the Wnt/calcium pathways, respectively. The two latter are β-catenin independent and represent examples of the non-canonical cascades. In this regard, a multitude of combinations between the 19 Wnt ligands and the 10 FZD receptors, such as co-receptors and other molecules, are theoretically possible. Classically, Wnt1/2/3/3a/8a/8b/10a/10b and FZD1/5/7/9 are classified as the canonical elements, whereas Wnt4/5a/5b/6/7a/7b/11 and FZD2/3/4/6 are designated as non-canonical components. The remaining Wnt2b/9a/9b/16 and FZD8/10 proteins remain unclassified [19,20]. However, it remains elusive how selectivity between Wnt/FZD as well as specificity of downstream signaling is achieved. Some Wnt/FZD elements can share dual canonical and non-canonical functions. For instance, it has been shown that in absence of Ror2 co-receptors, Wnt5a can activate β-catenin signaling with FZD4 and Lrp5 [21]. FZD3 has been described to act likely through canonical pathways in mice neurogenesis [21]. Zhang *et al.* demonstrated that in Xenopus foregut, FZD7 can activate low level of β-catenin and non-canonical JNK signaling in which both pathways contributed to foregut fate and proliferation while JNK pathway regulated cell morphology [22]. It is of interest that canonical and non-canonical pathways can not only be driven by specific Wnt/FZD combinations, but also by cell type, differentiation status, localization and composition of the microenvironment [23].

The canonical Wnt/FZD pathway

The β-catenin protein, encoded by the *CTNNB1* gene, is a key component of Wnt-canonical pathway signaling. β-catenin has a central region which presents armadillo domain repeats important for the binding of partners, such as Axin1 and adenomatous polyposis coli protein (APC) as well as transcription factors [24]. The C- and N-terminal regions are important. C-terminus of β-catenin serves as a binding factor for a multitude of complexes promoting β-catenin-mediated transcription, whereas phosphorylation of the N-terminus promotes degradation of β-catenin. Indeed, β-catenin may be present in several cellular compartments, such as the inner plasma membrane having a role in cell-cell junctions, the cytoplasm and the nucleus where it forms an active complex containing TCF/LEF transcription factors (T-cell factor/lymphoid enhancer factor) [25]. In the absence of nuclear β-catenin, TCF/LEF interact with the transcriptional co-repressor transducin like enhancer-1 (TLE-1) (Drosophila homolog Groucho), thus preventing β-catenin target gene expression [26]. Following translocation into the nucleus, β-catenin binds to TCF/LEF and replaces the TLE-1 repressor to form a transcriptional complex that activates the expression of its target genes (Fig. 1).

In absence of the canonical Wnt signaling, cytosolic β-catenin is targeted for degradation by a complex composed of a scaffold of proteins named axin1, APC, and two serine/threonine kinases: the glycogen synthase kinase 3β (GSK3β) and the casein kinase 1 (CK1) [27] (Fig. 1A). Axin1 and APC act together as scaffolding proteins through binding of β-catenin, and enhance its N-terminal phosphorylation by GSK3β and CK1. The first phosphorylation event is generated by CK1 at Ser45 which allows the GSK3β-mediated sequential phosphorylation of Thr41, Ser37, and Ser33 [28,29]. Ser37 and Ser33 phosphorylations provide a binding site for the E3 ubiquitin ligase β-TRCP (β-transducin repeat containing protein), leading to β-catenin ubiquitination in a β-TRCP/Skp1/cullin F-box complex (SCF) dependent manner followed by proteasomal degradation [30,31].

Activation of the canonical Wnt signaling cascade leads to disruption of the β-catenin degradation complex, resulting in β-catenin accumulation in the cytoplasm followed by translocation into the nucleus where it serves as a transcription factor to activate downstream target genes (Fig. 1B). In brief, this process is as