MINI REVIEW

The Wnt/β-catenin pathway as a therapeutic target in human hepatocellular carcinoma

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Summary Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide. However, targeted therapies are still at their beginning for the treatment of this poor-prognosis tumor. Among the signaling cascades deregulated in HCC, the Wnt/β-catenin signaling pathway plays a key role in hepatic oncogenesis. Although it has been shown, using HCC cell lines, that inhibition of the β-catenin signaling has anti-tumoral effect, no molecules targeting the Wnt pathway are currently tested in clinical trials for the treatment of HCC. Here we review our current knowledge about the role of the Wnt/β-catenin pathway in hepatocellular carcinoma pathogenesis and the benefits and limits of targeting this pathway in HCC.

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Introduction

Primary liver cancer is the sixth most common cancer and the third cause of cancer-related death worldwide [1]. Hepatocellular carcinoma (HCC) accounts for about 85% of primary liver cancers and its incidence has been increasing dramatically since 20 years. There are huge geographical variations in the incidence and risk factors. For instance, in Sub-Saharan Africa and in Asia, chronic viral hepatitis B is the main risk factor of HCC while in Europe and in the USA the incidence is lower, but continuously increasing as a result of higher prevalences of chronic viral hepatitis C, alcoholic cirrhosis and metabolic liver diseases [1]. The main curative treatment is surgery, but most patients have inoperable HCC. Until now, systemic therapies only demonstrated a limited impact on patient prognosis and sorafenib, the anti-angiogenic and Ras-Raf-MAPK inhibitor, is the single targeted molecule that has shown to significantly improve survival of patients with advanced HCC [2]. To develop more specific and targeted therapies, it is mandatory to understand the cellular and molecular mechanisms involved in the pathogenesis of HCC. Analyses of different genetic alterations have led to the identification of several major oncogenic pathways that are deregulated in HCC, including the p53, the Rb and the Wnt/β-catenin pathways and, to a lesser extent, the TGFβ and the MET-HGF pathways [3].

Mutations affecting the Wnt/β-catenin pathway appear to be the most frequent genetic event in human HCC [3]. Interestingly, recent data showed that the Wnt/β-catenin signaling pathway exert a critical role in many aspects of liver development and physiology [4—7]. It is tightly controlled by multiple factors and understanding such regulation opens a unique field for the development of targeted treatments. This review will discuss some of the potential

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molecular factors among these components that may be useful for the development of targeted treatments for HCC.

The Wnt/β-catenin signaling pathway in normal liver development and physiology

The Wnt/β-catenin pathway is a key developmental pathway also involved in the formation of multiple types of cancer, [8] for review. It controls tissue development in embryos and tissue maintenance in adults, through a genetic program induced in specific temporal, spatial and tissue contexts. This genetic program controls various critical cell processes such as cell proliferation, cell fate, epithelial-mesenchymal transitions and cell death and different biological responses are observed in the different tissues targeted by the Wnt/β-catenin pathway. Aberrant constitutive activation of the Wnt pathway leads to uncontrolled cell proliferation, growth and survival, promoting the development of cancer.

A general diagram of the Wnt pathway is shown in Fig. 1. The Wnt pathway is activated by the binding of secreted growth factors of the Wnt family to Frizzled (Fzd) receptors at the surface of target cells, leading to the activation of different signal transduction pathways: the canonical and non-canonical Wnt pathways, [9] for review. Herein, we exclusively focus on the canonical Wnt/β-catenin pathway, which is yet described to be the most relevant of both pathways in cancer biology. In the canonical Wnt signaling, β-catenin is the key transduction partner. Its concentration in the cytoplasm of unstimulated cells is kept very low through phosphorylation by two kinases, CK1 and GSK3β, functioning in a multiprotein complex including the products of two tumor suppressor genes, the adenomatous polyposis coli (APC) and Axins genes. Phosphorylated β-catenin is then ubiquitinylated, resulting in its proteasomal degradation. Accessibility of the Wnt ligands to the Fzd receptors is controlled by different Wnt inhibitors present in the extracellular compartment that prevent illegitimate β-catenin transduction signaling. In the activation state, when Wnt ligands bind to Fzd-LRP5/6 (Fzd-low-density lipoprotein receptor-related protein5/6) receptor complexes, a series of events preventing the cytosolic degradation of β-catenin occurs, inducing β-catenin accumulation in the cytosol and its translocation to the nucleus. β-catenin then interacts with the Tcf/Lef transcription factors to ensure the efficient regulation of Wnt target gene transcription. β-catenin is also involved in adherent junctions, in which it interacts with the cytoplasmic domain of E-cadherin. β-catenin is thus a multifunctional protein with both adhesive and transcriptional activation functions. The role of β-catenin in adhesion implies the membranous structural pool of this protein, whereas the Wnt signaling function of β-catenin involves the dynamic cytosolic and nuclear pools of the protein.

The Wnt/β-catenin pathway is a central regulatory system in normal liver cells. It is involved in all steps of liver development, including early specification, control of cell proliferation and control of the cell fate of the hepatoblast progenitor cells [4–6]. It is also essential for adult
liver homeostasis, being a master controller of the metabolic zonation within the liver lobule [7].

**Aberrant activation of the Wnt/β-catenin pathway in hepatocellular carcinoma**

Hepatocarcinogenesis is a multi-step process with accumulation of genetic and epigenetic events during tumor progression. In this context, the aberrant activation of the β-catenin signaling has been shown to play a major role in the pathogenesis of HCC.

Various molecular and genetic factors participate to such aberrant activation of the Wnt/β-catenin pathway (Fig. 2). Firstly, mutations are frequently identified in genes encoding for the main actors of the pathway. Gain-of-function mutations of CTNNB1 (encoding for β-catennin) are encountered in about one third of HCCs [10] and define CTNNB1 as the most frequently mutated gene in HCCs. Conversely, loss-of-function mutations of negative regulators of the pathway are also observed, namely mutations of the AXIN1 and AXIN2 genes (<5%) and of the APC gene (exceptional) [3]. Beside such mutational events, the Wnt/β-catenin pathway can be also activated in HCCs as a consequence of a deregulated dialogue between the tumor cells and their microenvironment. For instance, an autocrine Wnt stimulation loop can be established following epigenetic events that change the expression profiles of the ligands, the extracellular inhibitors and the receptors of the Wnt family [11]. A modified cross-talk with other signaling pathways such as HGF or TGF-β can also account for the aberrant activation of the Wnt/β-catenin pathway in HCCs [12,13].

Several studies based on genomic and transcriptomic profiling have led to the identification of two main sub-classes of HCCs [14,15]. Interestingly, these two subgroups of HCCs highlight the involvement of two different oncogenic pathways, which imply different oncogenic events and risk factors and define two distinct groups of patients with different outcomes. The first group comprises HCCs with high levels of chromosomal instability and frequent TP53 and AXIN1 mutations. This group is closely linked to an HBV history and a poor prognosis. The second group comprises HCCs with low genomic instability and frequent CTNNB1 mutations. This group is associated with a better patient prognosis [16].

Surprisingly, our works showed that CTNNB1- and AXIN1-mutated HCCs have very different transcriptional programs [17], explaining their opposite categorization within the molecular classification exposed above. We also showed that CTNNB1-mutated HCCs constitute a very homogeneous group of well-differentiated tumors with distinctive morphological, metabolic and molecular features [18]. Oppositely, other studies have shown that the Wnt/β-catenin pathway can also be activated in a subgroup of HCCs with progenitor features. Interestingly, such tumors are characterized by a low differentiation, a poor prognosis and the absence of CTNNB1 mutation [19]. Even if the molecular events that conduce to the activation of the Wnt/β-catenin signaling in such tumors are currently unknown, this subgroup of HCCs, as well as we observed for the AXIN1-mutated HCCs, displays a clearly divergent Wnt signature from the one of CTNNB1-mutated HCCs.

Altogether, these data suggest that if the activation of the Wnt/β-catenin pathway seems to be a recurrent event

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**Figure 2** The road towards cancer. The scheme represents three way of activation of the Wnt/β-catenin pathway, (A) by loss of function of the tumor suppressor genes, APC, AXIN, (B) by gain of function of CTNNB1, (C) by an autocrine mechanism resulting either from a loss of function of Wnt inhibitors, or up-regulation of Wnt ligands or receptors.
in HCCs, it appears to have very divergent molecular consequences according to the causative activating event. In our mind, the success of therapies that target the Wnt/β-catenin pathway relies on the definition of different working and robust Wnt signatures that would reflect the large heterogeneity of the HCCs phenotype according to the implied event that activates the Wnt/β-catenin pathway.

**Targeting the Wnt/β-catenin pathway in hepatocellular carcinoma**

In light of the different genetic events at the origin of an aberrant activation of the Wnt pathway, several strategies can be proposed:

- targeting the interaction between the Wnt ligand and the Fzd receptor;
- targeting the destruction complex;
- the most interesting but most difficult issue, targeting the β-catenin/Lef-Tcf transcriptional complex.

**Targeting the interaction between the Wnt/Fzd ligand/receptor complex**

Therapeutic monoclonal antibodies are useful for neutralizing extracellular targets such as ligands or extracellular domain of receptors. Therefore, Wnt and Fzd proteins are good candidates for this type of therapeutic approach, and should target HCCs that display an autocrine Wnt activation loop. Antibodies against Wnt-1, Wnt-2, Fzd-1 and Fzd-2 have been raised and showed some therapeutic impact in different tumoral contexts, [20] for review. Recently, an anti-Wnt-1 antibody was shown to inhibit the β-catenin signaling and induce apoptosis in two different HCC cell lines, and to decrease tumor growth when the cells are xenografted in nude mice [21]. Another strategy consists in the use of soluble Fzd receptors that will quench the Wnts molecules within the pericellular matrix. Soluble Fzd-8 was firstly shown to inhibit the tumor growth of the colorectal HTCl16 [22] and, recently, soluble Fzd-7 was also shown to inhibit Wnt signaling and to sensitize to doxorubicin treatment several HCC cell lines [23]. Finally, Dishevelled (Dvl), the molecular transducer of the Wnt/Fzd interaction, can also constitute an interesting target. In this optic, a competitive peptide inhibiting the Fzd/Dvl interaction has been designed and showed to decrease the β-catenin signaling and to induce apoptosis of the HuH7 cancer cell line [24].

**Targeting the β-catenin destruction complex**

Axin is one of the major components of the destruction complex. Transfection of HCC cell lines with adenoviruses encoding for Axin 1 inhibits cell proliferation and induce apoptosis [25]. Different studies, performed on colon cancer cells, have identified molecules that are able to inhibit the Wnt/β-catenin signaling by stabilizing Axin, leading to a reduced cell proliferation and viability [26,27]. These compounds may have a promising outcome in HCC therapy.

**Targeting the β-catenin/Lef-Tcf transcriptional complex**

The shared end-point of the aberrant activations of the Wnt/β-catenin signaling is the formation of β-catenin/ Lef-Tcf complexes in the nuclei that lead to the constitutive activation of a genetic program promoting cancer development. Thus, drugs designed to disrupt the Lef-Tcf binding of β-catenin appear to be the best approach for inhibiting cancer development induced by an aberrant activation of the Wnt pathway. Several molecules have been identified using high-throughput screening programs. In colon cancer cells, some molecules were identified as inhibitors of the interaction between Tcf4 and β-catenin, resulting in a reduced proliferation rate [28]. However, such experiments were counterbalanced by a much more limited impact on tumor growth in xenograft models [29]. It has been proposed that these molecules lack specificity and may also alter β-catenin-dependant cell adhesion [20]. Another molecule, which binds to the histone acetyltransferase CBP and could thus inhibit the transcriptional activity of β-catenin/lefr-Tcf complexes, has also been tested and was shown to promote apoptosis in colon cancer cells [30]. However the efficiency of these molecules in vivo remains to be evaluated. Furthermore, the potential toxicity of such inhibitors should be high regarding the broad range of CBP involvement on general transcriptional regulation.

In fact, although disruption of the β-catenin/Lef-Tcf complex is theoretically the best therapeutic strategy to inhibit HCCs associated to an aberrant Wnt signaling, the complexity of the transcriptional regulation of the Wnt target genes [31] suggests that much knowledge is still required before such drugs could be tested in clinical trials.

**Conclusion and perspectives**

Rationale to develop drugs that block the effects of constitutive Wnt/β-catenin signaling in HCCs is based on the knowledge of this signaling pathway, and drugs are designed to target different levels of the pathway. HCC with autocrine stimulation may be targeted with an antibody-based inhibition of the receptor-ligand interaction at the cell surface or with small inhibitors of essential upstream events such as the Fzd-Dvl complex formation. Drugs developed to block aberrant β-catenin/Lef-Tcf transcriptional activity have the better potential therapeutic effect, and are supposed to target HCC with either CTNNB1 or AXIN mutations. Moreover, it should be effective in the numerous cancers bearing a constitutive activation of the pathway. However, the complexity of the β-catenin-dependant transcription mechanisms makes the challenge ambitious. Furthermore, such drugs may have important side effects in organs such as intestine where the Wnt/β-catenin signaling is crucial for tissue renewal. The key for such strategy to reach the clinical practice is the identification of new molecules that would be effective only in tumor cell bearing an aberrant β-catenin signaling.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.
β-catenin signaling in hepatocellular carcinoma

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