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The Wnt/ β -catenin signaling pathway in liver biology and disease

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Abstract

The Wnt signaling pathway is an evolutionarily conserved, highly complex signaling pathway that is critical for development, differentiation and cellular homeostasis. The protein β -catenin is the central player in one major arm of the Wnt pathway called the canonical Wnt pathway. As in other organs, the Wnt/ β -catenin pathway is critical for liver development. However, recent research suggests that the pathway is also important in liver regeneration, liver metabolism and maintenance of normal function in the adult liver. Aberrant activation of β -catenin has also been implicated in the pathogenesis of hepatobiliary neoplasia, ranging from benign lesions to liver cancer. The explosion of research into the many roles of the Wnt/ β -catenin pathway promises to change our fundamental understanding of normal liver biology and the aberrations that lead to disease and cancer.

Keywords

ammonia detoxification; bile acid homeostasis; canonical Wnt signaling; cytochrome P450; hepatocellular carcinoma; liver biology; liver fibrosis; metabolic zonation; oxidative stress; Wnt/ β -catenin pathway

The Wnt pathway

The Wnt pathway is a highly conserved signaling pathway that is found in all phyla of the animal kingdom. The first member of the Wnt pathway was identified in the fruit fly *Drosophopila melanogaster* as the product of the Wingless gene (*wg*), mutations in which led to the frequent loss of the wing [1]. A few years later, Nusse and Varmus, while working on the mechanisms of oncogenesis of retroviruses, found that insertion of the mouse mammary tumor virus at the *int1* locus in the mouse genome turned on the proto-oncogene and led to tumor formation [2]. Subsequently, it was discovered that the mouse mammary oncogene *int-1* is an ortholog of the *Drosophila* Wingless gene [3]. That first member of the Wnt pathway is now called Wnt-1 (from Wg and int-1). Since those pioneering discoveries, there has been an explosion of research on the pathway's highly conserved nature, very

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complex regulation, multiple molecular interactions, and its role in cell fate determination, cell polarity and proliferation, as well as in cellular homeostasis.

The Wnts are a family of secreted glycoproteins and 19 Wnt ligands have been identified in mammals [4]. Intracellular signaling by Wnt ligands is transduced via two different pathways, the 'canonical pathway', which involves regulation of the protein β -catenin (called the Wnt/ β -catenin pathway) and the 'noncanonical pathway', which is independent of β -catenin (also called the Wnt/Ca or planar polarity pathway). While the two components of the Wnt pathway have generally been studied separately, recent evidence indicates that there is considerable cross-talk between the two pathways.

Canonical Wnt pathway

The canonical Wnt/ β -catenin pathway is centered on regulating the levels of its major effector β -catenin [5]. In the absence of Wnt ligands, β -catenin is sequestered in the cytoplasm by a protein complex composed of the scaffolding protein Axin, adenomatous polyposis coli (APC) gene product, CK1, and GSK3 β . Specific serine and threonine residues in the amino terminal region of β -catenin are sequentially phosphorylated by CK1 and GSK3 β . Phosphorylated β -catenin is then ubiquitinated and targeted for proteasomal degradation (reviewed in [6]). As a result of this continuous degradation, β -catenin is prevented from accumulating in the cytoplasm, and therefore from reaching the nucleus. In the absence of nuclear β -catenin, Wnt-target genes are repressed by binding of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of proteins (Figure 1A).

The canonical pathway is activated when Wnt ligands bind to the transmembrane Frizzled (Fz) receptor and the Fz-coreceptors low-density lipoprotein receptor-related protein (LRP) 5 or 6. The complex formed by Wnt-Fz-LRP5/6 is followed by recruitment of the scaffolding protein Dishevelled (Dvl), phosphorylation of LRP6 and recruitment of the axin degradation complex. These events prevent phosphorylation of β -catenin and result in its stabilization and cytoplasmic accumulation. β -catenin then translocates to the nucleus where it forms a complex with the TCF/LEF transcription factors and activates target gene expression (Figure 1B; reviewed recently in [7]).

A recent study identified several Wnt ligands that were expressed by various liver cells, including hepatocytes, Kupffer cells, stellate cells, sinusoidal endothelial cells and biliary epithelial cells, suggesting a high degree of complexity of Wnt signaling and potential cross-talk between different cell types in the liver [8]. In addition, lipoprotein particles and glypicans can act as vehicles for the movement of Wnt ligands to distant sites, suggesting that intracellular Wnt signaling can be modulated by systemic signals [9,10].

In addition to its transcriptional functions, β -catenin also participates in cell-cell adhesion via its association with E-cadherin at the cell membrane [11]. The transmembrane E-cadherin molecules mediate homotypic cell-cell contact. β -catenin binds to E-cadherin and also to α -catenin. The latter protein interacts with the actin cytoskeleton in a dynamic fashion, a process that is regulated by the binding of α -catenin to β -catenin [12,13]. It was recently found that the two pools of β -catenin, one involved in transcriptional activation of Wnt-target genes, and the second involved in cadherin-based adherens junctions, are integrated. Dissociation of adherens junctions led to internalization of the cadherin-bound pool of β -catenin and its accumulation at the perinuclear endocytic recycling compartment, from where it translocated to the nucleus on activation of the Wnt pathway [14].

A third interaction of β -catenin relevant to Wnt signaling, particularly in the liver, is the association of β -catenin with c-Met, the receptor for HGF. In the presence of HGF, the c-Met- β -catenin complex dissociates, there is phosphorylation of β -catenin at specific

tyrosine residues and β -catenin can translocate to the nucleus and induce mitogenesis [15,16].

Noncanonical Wnt pathways

The noncanonical Wnt pathways are defined as signaling initiated by Wnt or Fz that takes place independently of β -catenin transcriptional function [17]. These pathways are not as well studied compared with the canonical pathway, and consist of several pathways that intersect or overlap. Among the pathways that have been described are the Wnt/calcium signaling pathway that regulates intracellular calcium flux via G protein, Wnt/planar cell polarity pathway that activates trimeric G proteins and Dvl and regulates actin cytoskeleton and cell adhesion, Wnt/PKA signaling that is implicated in myogenesis, and many others. The remainder of this article will focus on the canonical Wnt pathway, and the reader is referred to several recent reviews on the noncanonical Wnt signaling pathways [18–20].

The many roles of Wnt/ β -catenin signaling in liver biology

Since the discovery in 1980 by Christiane Nüsslein-Volhard and Eric Wieschaus (winners, along with Edward B Lewis, of the Nobel Prize in Physiology or Medicine in 1995 for their work on genetic control of embryonic development) that Wnt was a segment polarity gene in the fruit fly [21], the Wnt/ β -catenin pathway has been intensely studied in prenatal development, hepatic fate specification of stem cells and liver organogenesis [22]. However, in addition to its role as a critical regulator of liver development, the Wnt/ β -catenin pathway has emerged as a key player in many aspects of postnatal liver homeostasis. Recent work has highlighted the fact that the pathway is closely associated with many of the most important functions of the liver, such as ammonia and nitrogen metabolism, bile acid homeostasis and drug detoxification. In addition, the pathway is also important for helping the liver resist and recover from toxic and metabolic injury and oxidative stress, and its aberrant activation can lead to benign and malignant tumors of the liver (Figure 2). This article will focus on the many emerging roles of the canonical Wnt/ β -catenin pathway in adult liver biology. The reader is referred to the many excellent reviews that have discussed the role of the Wnt/ β -catenin pathway in prenatal liver development [22–25].

Role of the Wnt/ β -catenin pathway in postnatal liver growth & regeneration

While there is little doubt that β -catenin is essential for embryonic liver development, its role in postnatal liver growth and regeneration appears to be important but not indispensable. Two groups independently reported that hepatocyte-specific disruption of β -catenin in mice resulted in decreased liver size along with associated decrease in hepatocyte proliferation, but the mice had normal body weight and normal life spans [26,27].

Complementary studies with mice overexpressing β -catenin in the liver demonstrated that there was increased hepatocyte proliferation and an increase in liver size [28].

β -catenin also appears to play a supportive, but not essential, role in liver regeneration. The protein exhibits rapid nuclear translocation in the two-thirds partial hepatectomy model of liver regeneration in rats, loss of β -catenin results in delayed liver regeneration [26], and expression of a stable, mutated form of the protein causes accelerated regeneration [29].

The relevance of the role of β -catenin in liver regeneration has been established recently in an animal model of acetaminophen-induced liver injury and retrospective analysis of patients with acetaminophen-induced hepatotoxicity [30]. Administration of acetaminophen at a dose that was nonlethal and sufficient to induce liver regeneration after injury caused stabilization and activation of β -catenin and increased expression of its target gene glutamine synthetase (GS). β -catenin knockout mice demonstrated decreased liver

regeneration after acetaminophen-mediated liver injury, while a positive correlation was found between nuclear β -catenin and spontaneous liver regeneration in patients with acute liver injury resulting from acetaminophen overdose. Taken together, studies evaluating the role of β -catenin in liver regeneration suggest that the presence of β -catenin promotes liver regeneration, and its absence results in a suboptimal regenerative response.

Role of β -catenin in establishing hepatic metabolic zonation

While the liver lobule appears to be histologically homogeneous, in reality hepatocytes exhibit a striking functional heterogeneity. Nearly 30 years ago, the pioneering work of Jungermann and colleagues established that several metabolic processes in the liver are arranged in a gradient [31,32]. Normally, the blood enters the hepatic lobule via the terminal branches of the portal vein and the hepatic artery and travels across the hepatic sinusoid towards the central vein. Hepatocytes in the area of the portal triad (periportal or zone 1 hepatocytes) are functionally distinct from the hepatocytes around the central vein (perivenous or zone 3 hepatocytes).

In the liver, gluconeogenesis, cholesterol biosynthesis and urea synthesis predominate in the periportal zone, while the opposing metabolic functions of glycolysis, bile acid biosynthesis and glutamine synthesis are most prominent in the perivenous zone. Interestingly, some functions (e.g., carbohydrate metabolism) exhibit a dynamic pattern of zonation with contraction or expansion depending on the metabolic state [33]. On the other hand, some functions (e.g., ammonia detoxification) show strict zonation, with expression of GS restricted to a small rim of perivenous hepatocytes that in turn do not express the urea synthetic enzyme carbamoyl phosphate synthase (CPS) (Figure 3) [34].

While the zonal distribution of metabolic function in the liver was known for several decades, only recently did the Wnt/ β -catenin pathway emerge as the master regulator of hepatic zonation, after it was found that expression of GS, a marker of perivenous hepatocytes, was upregulated in tumors with activating β -catenin mutations [33,35,36]. Direct proof of perivenous gene regulation by β -catenin was provided by the use of *APC* gene knockout mice with aberrant activation of β -catenin, which exhibited an extension of the normally perivenous zone of GS expression towards the portal region [37]. These results were complemented by studies using β -catenin knockout mice that demonstrated that liver-specific loss of β -catenin resulted in loss of GS expression from perivenous hepatocytes, providing further proof of the role of β -catenin in zonal hepatic gene expression [27,38].

Recently, it was further established that liver zonation is established by β -catenin irrespective of the presence of its regulator APC and also independently of its downstream target c-Myc [39]. Additional insight was proved by Colletti *et al.* using an innovative approach of transcriptional profiling of hepatocytes derived from *in vitro* differentiation of stem cells, who demonstrated that an interplay between the Wnt/ β -catenin pathway and the nuclear transcription factor hepatocyte nuclear factor-4 α (HNF4 α) determines the zonal phenotype of hepatocytes [40]. Spontaneous differentiation of liver stem cells gives rise to hepatocytes with a periportal phenotype. However, the phenotype switches to the perivenous type after activation of the Wnt pathway using an inhibitor of GSK3 β . Interestingly, this switch from periportal to a perivenous phenotype occurs as a result of the binding of the LEF1 transcription factor (a member of the Wnt signaling pathway) to its own binding sequence, as well as that of HNF4 α . As a result of this interaction, HNF4 α is displaced from the promoter region of its target genes leading to repression of periportal gene expression, while binding of LEF1 to promoters of perivenous target genes activates their expression, thereby affecting the phenotypic switch [40]. While many questions regarding the precise molecular regulation of hepatic metabolic zonation remain, the identification of β -catenin as

one of the key players in this process represents a significant advance in the understanding of this important feature of normal liver physiology.

Regulation of ammonia detoxification by the Wnt/ β -catenin pathway

The role of β -catenin in regulating ammonia detoxification in the liver directly results from its importance in establishing hepatic metabolic zonation. Two systems are at play during ammonia removal by the liver. Hepatocytes in the periportal zone express enzymes of the urea cycle. CPS-1 is the rate-limiting step of the urea cycle. On the other hand, hepatocytes in the perivenous zone express GS that catalyzes the formation of glutamine from glutamate and ammonia. While the urea cycle is a high-capacity/low-affinity system, GS is a low-capacity/high-affinity system. Together, the two systems effectively remove ammonia, since any ammonia that is not taken up by the urea cycle in the periportal and intermediate regions is scavenged by perivenous hepatocytes [34].

As discussed previously, aberrant activation of β -catenin results in extension of the normally perivenous pattern of expression of GS towards the periportal area. Concomitantly, there is loss of periportal expression of CPS I. By contrast, loss of β -catenin abolishes GS expression from perivenous hepatocytes. The functional significance of the perivenous GS expression was highlighted by the finding that, while β -catenin knockout mice demonstrated normal blood ammonia levels on a standard diet, there was marked increase in blood ammonia levels in mice fed with a high-protein diet [38].

Drug detoxification & cytochrome P450 enzyme regulation by β -catenin

The cytochrome P450 (CYP) family is a large group of hemethiolate monooxygenase enzymes that plays an important role in the metabolism of xenobiotics such as drugs, carcinogens and mutagens [41]. CYPs exhibit a tissue-specific expression pattern, and some members of the family are constitutively expressed, while others are inducible by transcriptional or post-transcriptional mechanisms by several ligand-dependent transcription factors, such as constitutive androstane receptor, pregnane X receptor and aryl hydrocarbon receptor [42].

The initial clue suggesting a role for β -catenin in regulating CYP gene expression came from studies using a rodent model of carcinogenesis using phenobarbital as a tumor promoter. Mice treated with the liver carcinogen *N*-nitrosodiethylamine (DEN) in the presence of phenobarbital exhibited a high percentage of tumors with mutations in the β -catenin gene (*Ctnnb1*). Tumors with activating β -catenin mutations also exhibit expression of a downstream β -catenin target GS. Loeppen *et al.* demonstrated that liver tumors in mice with GS expression also express CYP1A, CYP2B, CYP2C and CYP2E1, while tumors that did not contain *Ctnnb1* mutations exhibited low expression levels of these CYPs. Further proof of transcriptional activation of CYP genes by β -catenin was provided by cotransfection studies using a constitutively active mutated form of β -catenin [43]. Subsequently, two groups independently demonstrated that hepatocyte-specific loss of β -catenin results in decreased expression of several CYP family members [27,38]. Interestingly, β -catenin knockout mice exhibited an increased resistance to acetaminophen-induced liver injury as a result of decreased CYP2E1 expression in hepatic microsomes [27,38].

As far back as 1978, Gooding *et al.* demonstrated by microspectrophotometric analysis that perivenous (zone 3) cells contained up to twice as much cytochrome P450 as periportal (zone 1) cells in rat liver [44]. Bühler *et al.* subsequently demonstrated using immunostaining of consecutive thin liver section and western blot analysis that several CYPs, such as CYP2B1/2, CYP2E1 and CYP3A1, were expressed in a 6–8 cell thick layer

surrounding the terminal hepatic venules [45]. Since β -catenin plays an important regulatory role in perivenous gene expression as discussed previously, these findings raised the question of whether the zonal expression pattern of CYPs was also dependent on β -catenin. Analysis of tumors with activating Ha-ras or β -catenin mutations revealed that whereas tumors with *Ha-ras* mutations had lower expression of perivenous CYPs, tumors with β -catenin mutations exhibited reciprocal regulation with increased expression of perivenous CYP2E1 and CYP2C [46]. Further direct evidence of this zonal regulation of CYP expression by β -catenin was provided by a recent study showing that, in addition to regulating the basal expression levels, β -catenin also regulates the magnitude of induction and hepatic localization of CYPs by xenobiotic inducers [47]. In addition to regulating expression of Phase I enzymes of the CYP family, as discussed earlier, β -catenin also appears to be important for the activation and zonal expression of several members of the glutathione-*S*-transferase family, which are important Phase II drug-metabolizing enzymes [48]. Thus, β -catenin appears to have an important role in xenobiotic metabolism and drug toxification that opens up the exciting possibility of therapeutic modulation of Wnt signaling in the treatment of drug toxicity and drug-induced liver injury.

Role of β -catenin in biliary homeostasis & bile canalicular morphogenesis

Circumstantial evidence for a role of the Wnt/ β -catenin pathway in bile secretion and bile acid homeostasis came from several studies. First, it has been known for many years that CYP7A1 and CYP27, the key enzymes in bile acid biosynthesis, have a perivenous pattern of expression in the hepatic lobule [49]. Second, Audard *et al.* demonstrated that human hepatocellular carcinomas (HCCs) with mutations in β -catenin exhibit striking cholestasis [50]. Third, it was recently found that feeding β -catenin knockout mice the steatogenic methionine-choline-deficient diet led to accumulation of hepatic cholesterol and high serum bilirubin levels [51]. Taken together, these findings suggested that β -catenin played a heretofore unsuspected role in one of the liver's important functions, bile acid secretion and homeostasis.

Direct evidence for a role of β -catenin in bile acid homeostasis came from the finding that liver-specific β -catenin knockout mice exhibited intrahepatic cholestasis and decreased bile flow rates [52]. Furthermore, knockout mice exhibit altered staining for F-actin in the subcanalicular region, and by scanning electron microscopy were shown to have dilated, tortuous bile canaliculi with paucity of microvilli on the canalicular surface of hepatocytes (Figure 4). While knockout mice exhibit relatively mild cholestasis in the unstressed state, on being fed cholic acid there was striking increase in serum and hepatic bile acid levels, suggesting a profound defect in the adaptive response to bile acid load. Loss of β -catenin also abrogated expression to nearly undetectable levels of claudin-2, a protein that participates in the formation of intercellular tight junctions and regulates paracellular permeability [53]. While no obvious defect in tight junction morphology was found by electron microscopy, it is intriguing that loss of claudin-2 has itself been shown to cause bile canalicular morphogenesis defects [54]. In further support for a role of β -catenin in bile canalicular morphogenesis is the finding that depletion of β -catenin-based adherens junctions leads to defects in hepatocytes apical lumen remodeling [55]. Future studies should shed light on the precise molecular events and protein-protein interactions that β -catenin participates in to maintain bile acid homeostasis.

Regulation of hepatic vitamin C biosynthesis by the Wnt/ β -catenin pathway

While studying gene expression in liver-specific β -catenin knockout mice, Nejak-Bowen *et al.* recently discovered an interesting role for β -catenin in vitamin biosynthesis in the murine

liver [56]. They identified the gene *regucalcin* as a downstream target of β -catenin. Interestingly, *regucalcin*, also called senescence marker protein 30, is a gluconolactonase that plays a role in vitamin C biosynthesis [57]. Besides *regucalcin*, livers of β -catenin knockout mice were also found to be deficient in expression of L-gulonolactone oxidase, the final and rate-limiting step in ascorbate biosynthesis. Consistent with these findings, knockout mice maintained on chow diet were found to have over threefold lower serum ascorbate levels than wild-type mice [56]. While the significance of these findings to human disease is unclear, since humans have lost the ability to synthesize vitamin C, the loss of vitamin C biosynthesis may increase the susceptibility of murine hepatocytes to oxidative stress.

Role of the Wnt/ β -catenin pathway in the pathogenesis of hepatobiliary neoplasia

Since the discovery in 1993 that the human APC protein, which was known to be involved in the pathogenesis of colon and gastric cancers, associates with β -catenin [58], over 5000 research articles have been published on the involvement of β -catenin in various types of cancers. Therefore, it is not surprising that the role of the Wnt/ β -catenin pathway in the pathogenesis of hepatobiliary neoplasia has also received intense scrutiny. Only some salient features and recent advances in the understanding of the Wnt/ β -catenin pathway in liver tumors will be highlighted here, and the reader is referred to several recent comprehensive reviews on this topic [59–61].

Hepatocellular carcinoma & the Wnt/ β -catenin pathway

In 1996, Ihara *et al.* first demonstrated changes in the immunostaining patterns of β -catenin and β -catenin-associated cell adhesion molecules in HCCs [62]. Subsequently, de La Coste *et al.* demonstrated that approximately a quarter of human HCCs had β -catenin point mutations and deletions that led to aberrant β -catenin activation [63]. In addition, β -catenin mutations were also found in hepatoma cell lines and in a subset of transgenic mouse lines of HCC. Several studies have confirmed these initial findings and shown that point mutations at specific serine and threonine residues in exon 3 are important for the aberrant activation of β -catenin in a subset of human HCCs, although additional point mutations and deletions are also associated with similar activation (reviewed in [59]).

Given the complexities of the Wnt pathway and the multiple regulatory inputs and molecular interactions that can modulate its signaling, it is not surprising that many studies have shown that up to 90% of HCCs have aberrant β -catenin activation in the absence of activating mutations in the *Ctnnb1* gene. In these tumors, additional mechanisms such as the upregulation of Wnt ligand expression, increased expression of the FZ receptors, methylation of secreted FZ-related proteins, mutations in components of the β -catenin destruction complex such as axin1, and tyrosine-phosphorylation-dependent activation of β -catenin are responsible for activation of β -catenin [64–66]. A recent study found that the canonical Wnt3 and downstream β -catenin-target genes were upregulated in hepatitis B virus-induced liver cancers. Furthermore, it was shown that this upregulation of the Wnt pathway by Wnt3 was mediated by FZ-7 receptor (FZD7), since Wnt3 and FZD7 were found to interact in co-immunoprecipitation studies and activation of the Wnt pathway in a Wnt3-overexpressing cell line was abolished by siRNA-mediated FZD7 knockdown [67].

Whether the presence of Wnt pathways activation affects the biological behavior of HCC lesions and affects clinical outcomes is still unclear. Some studies have found association of β -catenin mutations or activation with worse outcomes, larger tumor size and increased vascular invasion [68–71]. On the other hand, other groups have reported that tumors

harboring β -catenin mutations are less invasive, have lower rates of portal vein involvement and better prognosis [72,73]. These apparently paradoxical findings may have an intriguing explanation involving autoregulation of Wnt signaling, as suggested by a recent study that used several HCC cell lines [74]. It was found that canonical Wnt signaling was activated in well-differentiated HCC cells, while noncanonical Wnt signaling was activated – and canonical signaling repressed – in poorly differentiated cells. Interestingly, expression of the noncanonical Wnt5a, which is known to inhibit canonical Wnt signaling, was increased in poorly differentiated HCC cell lines. When Wnt5a was expressed in a well-differentiated cell line that normally shows high canonical Wnt activation due to an activating β -catenin mutation, repression of canonical Wnt signaling was found. Based on these results, the authors proposed that the two Wnt pathways are selectively activated or repressed based on the differentiation status of HCC cells, and have complementary roles in hepatocarcinogenesis. In this model, canonical Wnt signaling contributes to tumor initiation and noncanonical Wnt signaling to tumor progression [74]. In addition to Wnt5a, the noncanonical Wnt11 was recently found to have a role as a cancer suppressor, providing further proof of a complex interplay between the canonical and noncanonical Wnt signaling pathways. Expression levels of Wnt11 were lower in human HCC tumors compared with adjacent liver tissue, and overexpression of Wnt11 reduced cell motility and migration of liver cancer cell lines via activation of Rho kinase [75].

In addition to the controversy regarding the clinical correlates of Wnt pathway activation in HCC, the precise role of the pathway in the molecular pathogenesis of liver cancer is also uncertain. In support of a direct role of β -catenin in hepatocarcinogenesis was the finding that aberrant activation of β -catenin by inactivating its suppressor APC led to the spontaneous development of HCCs in mice [76]. On the other hand, mice overexpressing either mutated or non-mutated forms of β -catenin did not develop spontaneous tumors [28,77,78]. Recently, it was shown that mice expressing a mutated form of β -catenin harboring a mutation at the serine-45 residue in exon 3 did not develop spontaneous tumors, but had increased susceptibility to developing HCCs on exposure to the carcinogen DEN [29]. Interestingly, mice with liver-specific disruption of β -catenin also showed increased DEN-induced hepatocarcinogenesis via increased oxidative stress and hepatocyte proliferation resulting from PDGFR α /PIK3CA/Akt activation and c-Myc overexpression [79]. Taken together, these findings argue against the simple model of a direct oncogenic role of the Wnt/ β -catenin pathways in hepatocarcinogenesis. Rather, these results suggest that the role of the pathway in the development of liver cancer is highly context-dependent and likely to be related to its role in cellular homeostasis. Also, Lucero *et al.* recently proposed a similar complex role of the Wnt/ β -catenin pathway in the pathogenesis of malignant melanoma [80]. While it has been shown that pharmacologic inhibition of β -catenin decreases survival of hepatoma cells [81], given the inherent complexity of Wnt/ β -catenin signaling in the liver, further research is needed to fully understand the implications of therapeutic inhibition of the pathway in HCC.

Hepatoblastomas & the Wnt/ β -catenin pathway

While Wnt/ β -catenin mutations have been implicated in approximately a third of all HCCs, in the case of the most common childhood hepatic malignancy, hepatoblastoma, mutational activation of β -catenin is found in 50–90% of tumors [82–84]. Hepatoblastomas contain various amounts of epithelial cells as well as mesenchymally derived components, features on which histopathological classifications have been based. Based on the level of differentiation of the cells, epithelial-type hepatoblastomas have been further classified as embryonal subtype, which contains more immature cells, the fetal subtype, which contains more differentiated clusters of hepatocytes, as well as microtrabecular and undifferentiated subtypes [85]. Recently, using microarray analysis, Cairo *et al.* identified two subclasses of

hepatoblastomas that resemble distinct stages of liver development. Most poorly differentiated hepatoblastomas had gene-expression profiles that resembled mouse livers at early stages of embryonic development, whereas differentiated tumors were similar to mouse livers at late fetal and postnatal stages [86]. While mutational activation of β -catenin was found in both subtypes, poorly differentiated tumors demonstrated more nuclear accumulation and decreased membranous localization of β -catenin. Furthermore, while the well-differentiated (fetal-type) hepatoblastomas showed increased expression of Wnt-target genes such as GS that are expressed in mature hepatocytes, poorly differentiated (embryonal-type) tumors expressed the subset of Wnt-target genes involved in cell proliferation and survival. Thus, hepatoblastomas exhibit context-dependent Wnt-target gene expression. These findings have clinical significance, since the expression signature of the two subtypes could predict disease outcome and therefore identify high-risk patients.

Hepatic adenomas & β -catenin

Hepatic adenomas are benign tumors of the liver that generally present as solitary masses with a pseudocapsule from compression of adjacent normal liver tissue. Hepatic adenomas may undergo malignant transformation and have increased risk of hemorrhage. Adenomas have an association with the use of estrogen and androgen therapy, and may regress after the hormonal exposure is discontinued. However, given the potential for the complications mentioned earlier, resection is generally recommended for hepatic adenomas. A major subset of hepatic adenomas exhibits biallelic inactivation of the nuclear transcription factor HNF1 α . Another group of adenomas has mutations in the β -catenin gene or exhibit β -catenin activation without associated gene mutations [87]. A third group of hepatic adenomas exhibits inflammatory infiltrates and enhanced IL-6 signaling owing to somatic gain-of-function mutations in the IL-6 coreceptor gene encoding gp130 [88]. These important insights into the molecular pathogenesis of hepatic adenomas have important clinical implications, since adenomas with HNF1 α mutations have low potential for malignant transformation, while those with β -catenin mutations have higher propensity for turning malignant. Furthermore, β -catenin and gp130 mutations may both be present in HCCs, suggesting cooperation of these pathways in the malignant transformation of inflammatory adenomas [88].

Activation of β -catenin in focal nodular hyperplasia

Focal nodular hyperplasia (FNH) is a benign lesion of the liver that is characterized by hepatocyte hyperplasia in the setting of vascular malformations. Most FNH lesions show polyclonal expansion of hepatocytes resulting from hyperperfusion of the hepatic parenchyma. It was recently shown that FNH lesions have a characteristic pattern of GS staining, suggesting activation of the β -catenin pathway [89]. However, this β -catenin activation occurred without activating mutations in the *Ctnnb1* gene. Furthermore, the GS-staining pattern in FNH has been proposed as a reliable means to differentiate FNH lesions that rarely require therapeutic intervention from other lesions such as adenomas that do require treatment [90].

Role of β -catenin in protection against oxidative stress in the liver

While the role of β -catenin in development and carcinogenesis has been studied for 20 years, new insight into another critical function of β -catenin was provided by the discovery of an evolutionarily conserved functional interaction between β -catenin and forkhead box O (FOXO) transcription factors in response to oxidative stress [91]. When cells are exposed to low levels of oxidative stress, interaction of β -catenin with FOXO transcription factors is stimulated leading to enhanced FOXO activity, inhibition of cell-cycle progression and increased resistance to oxidative stress.

Another novel mechanism of regulation of the Wnt/ β -catenin pathways by the cellular redox state was discovered shortly thereafter by Funato *et al.*, who demonstrated an interaction between nucleoredoxin, a thioredoxin-like protein, and the cytoplasmic protein Dishevelled, which regulates GSK3 β -mediated β -catenin phosphorylation [92]. Nucleoredoxin is a strong inhibitor of Wnt/ β -catenin signaling. When cells are exposed to hydrogen peroxide to generate oxidative stress, interaction between nucleoredoxin and Dishevelled is inhibited, which results in increased Wnt pathway activity and stabilization of β -catenin.

Recently, it was shown in osteoblast precursor cells and in colon cancer cells that oxidative stress antagonizes Wnt signaling by diverting β -catenin from T-cell factor activity to FOXO transcription factors [93]. These findings further highlight the central regulatory role of β -catenin in cellular oxidative stress response and, given the highly conserved nature of Wnt/ β -catenin signaling, make it likely that similar adaptive mechanisms are active in the liver. Indeed, it was recently found that liver-specific β -catenin knockout mice exhibit significantly increased liver injury when fed the methionine-choline-deficient diet that is known to cause severe oxidative stress in the liver [51], further supporting an important role for β -catenin in hepatic adaptation to oxidative stress.

The Wnt pathway in hepatic fibrogenesis

Hepatic stellate cells synthesize the extracellular matrix proteins that lead to liver fibrosis and, ultimately, to cirrhosis. Using DNA microarrays to study gene-expression differences between quiescent and activated rat hepatic stellate cells, the ligands Wnt4 and Wnt5 and the Wnt receptor FZ-2 were found to be upregulated [94]. However, no changes were found in the phosphorylation state or nuclear translocation of β -catenin, suggesting that the noncanonical Wnt pathway was involved in the process of hepatic stellate cell activation.

In a subsequent study, increased expression of both canonical Wnt3a and 10b and noncanonical (Wnt4 and Wnt5a) Wnt genes, along with FZ-1 and -2 as well as FZ coreceptors, was found in activated stellate cells, but not in quiescent hepatic stellate cells [95]. However, higher levels of nuclear β -catenin and TCF DNA binding were found in activated HSC, suggesting that canonical Wnt signaling was also important in hepatic stellate cell activation. Since treatment with the inhibitors of Wnt signaling, Dkk-1 and Chibby, could restore quiescence of hepatic stellate cells in culture, and high expression of Dkk-1 could induce apoptosis of cultured stellate cells, these results raise the exciting possibility that blocking Wnt signaling could be a potential therapeutic strategy in preventing liver fibrosis.

Expert commentary & five-year view

Every passing year brings more surprises from the Wnt/ β -catenin pathway in liver biology. If one looks only at the bland histology of the liver lobule and the faint, uninteresting immunostaining for β -catenin at the periphery of hepatocytes in the adult liver, it is easy to be lulled into thinking that the Wnt/ β -catenin pathway is mainly a 'prenatal' signaling pathway with only a minor role in the adult liver. However, the literature is rapidly expanding, with examples of the remarkable diversity and complexity of functions fulfilled by β -catenin and other members of the Wnt pathway in normal liver biology. The pathway appears to be required by the liver to not only fulfill its major functions, such as metabolism, drug detoxification and bile secretion, but also for resisting metabolic and oxidative stress and for regenerating from injury to restore normal functioning.

Recently, Gebhardt and Hovhannisyan proposed a model to explain how β -catenin may regulate diverse processes in the liver and how its aberrant activation can lead to tumorigenesis [96]. According to this model, when signaling through the Wnt pathway is

occurring at low levels, β -catenin in the nucleus allows only high-affinity transcription factors to bind to it, leading to differentiation and formation of mature hepatocytes. At intermediate levels of activity, which is hypothesized to occur in the adult liver, β -catenin can bind to multiple transcription factors of approximately equivalent affinities, allowing this complex network of factors to finely regulate metabolic processes, including hepatic metabolic zonation. Any aberration occurring within this network could potentially contribute to the development of metabolic diseases. Finally, at the highest level of Wnt activity, high levels of nuclear β -catenin would be available to bind to and saturate its multiple binding partners, as well as to low-affinity factors, leading to cell proliferation and development of tumors. While an attractive model to explain the transcriptional functions of β -catenin, it remains to be seen how much the additional complexities of noncanonical Wnt signaling and its cross-talk with the canonical Wnt pathway, the role of the β -catenin pool that exists with the cell-adhesion complex, and the multiple feed-back loops that are operational within Wnt signaling influence this basic operational framework.

An inherent drawback with studies based on *in vivo* animal models, especially for complex pathways such as the Wnt signaling pathway, is that compensatory changes taking place within an organ or organism make interpretation of primary effects difficult. On the other hand, *in vitro* models suffer from the absence of microenvironmental cues that are so critical for regulating function within the liver. These methodological challenges and others, such as the relevance of animal models of Wnt/ β -catenin signaling to human liver function and diseases, will need to be carefully evaluated in the future. Along the same lines, while attempts have been made to treat liver cancers with anti-Wnt/ β -catenin therapy, given the many important roles played by the pathway in normal hepatocytes, careful consideration will have to be given to avoiding toxicity with such treatments.

Over the next 5 years, it is hoped that many remaining intriguing questions will be answered, and almost certainly, new ones raised regarding the complex biology of Wnt/ β -catenin in the liver. Whatever the answers to these questions turn out to be, it is certain that the Wnt/ β -catenin pathway will keep investigators busy trying to unravel its many mysteries for years to come.

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Key issues

- The Wnt pathway is a highly conserved signaling pathway that is found in all metazoans.
- Canonical Wnt signaling involves the regulation of the protein β -catenin, which has roles in transcriptional regulation, as well as cell–cell adhesion via its association with E-cadherin at the cell membrane.
- Regulation of the Wnt/ β -catenin pathway is complex, with inputs from multiple Wnt ligands, regulation of the canonical Wnt pathway components at several levels and multiple molecular interactions of β -catenin.
- In addition to well-established roles in liver development, the Wnt/ β -catenin pathway is emerging as an important regulator of multiple processes in the adult liver.
- β -catenin appears to play an important, but not essential, role in liver regeneration.
- The liver lobule exhibits metabolic zonation, with hepatocytes in the periportal and perivenous regions of the hepatic lobule exhibiting differing metabolic functions, and β -catenin is the master regulator of the perivenous pattern of gene expression.
- β -catenin regulates ammonia detoxification by regulating glutamine synthetase and cytochrome P450 enzymes involved in xenobiotic metabolism, as well as vitamin C biosynthesis.
- The Wnt/ β -catenin pathway is important for adaptation to oxidative stress, and loss of β -catenin in the liver increases susceptibility to diet- and alcohol-induced liver injury.
- Recent evidence suggests an important role of β -catenin in bile acid homeostasis and bile canalicular morphogenesis.
- The Wnt/ β -catenin pathway, as well as β -catenin-independent noncanonical Wnt signaling, has been implicated in the pathogenesis of a subset of liver cancers, as well as benign liver tumors.
- Many questions remain regarding the precise molecular role and regulation in the liver of the Wnt/ β -catenin pathway, and future research is likely to yield more surprising answers regarding this complex signaling pathway.

Figure 1. Overview of the canonical Wnt/β catenin pathway

(A) In the absence of Wnt signaling and in the presence of inhibitors such as Dkkopf, β-catenin is complexed with the degradation complex consisting of Axin, adenomatous polyposis coli protein and glycogen synthase kinase 3β. Phosphorylation of β-catenin by CK and GSK3β targets β-catenin for proteasomal degradation. At the cell membrane, β-catenin participates in the formation of adherens junctions by binding to E-cadherin, and also binds to the hepatocyte growth factor receptor Met (not shown). (B) Wnt signaling is activated by binding of the Wnt ligand to its receptor Frizzled and coreceptor low-density lipoprotein receptor-related protein 5/6, which recruits the protein disheveled and the β-catenin degradation complex. As a result, β-catenin remains unphosphorylated and accumulates in the cytoplasm and is available for translocation to the nucleus. In the nucleus, it binds to the T-cell factor/lymphoid enhancer factor family of transcription factors to activate target gene expression.

APC: Adenomatous polyposis coli protein; LRP: Lipoprotein receptor-related protein; TCF: T-cell factor.

Figure 2. The many roles of the Wnt/ β -catenin pathway in the liver

The pathway plays a critical role in early liver development, postnatal growth and regeneration. It is also important for many functions in the adult liver and its aberrant function has been implicated in the development of tumors.

Figure 3. Concept of hepatic metabolic zonation

Hepatocytes within the liver lobule exhibit functional heterogeneity with many metabolic functions arranged in a gradient. Hepatocytes closest to the portal triad (zone 1 or periportal) are mainly involved in oxidative energy metabolism, gluconeogenesis and urea synthesis. Hepatocytes closest to the central vein (zone 3 or perivenous) are involved in glycolysis, glutamine synthesis and xenobiotic metabolism. The intermediate zone is called zone 2. Metabolic zonation is dynamic for most functions, but strictly compartmentalized for expression of glutamine synthesis.

BD: Bile duct; CV: Central vein; HA: Hepatic artery; PV: Portal vein.

Figure 4. Disruption of β -catenin in the adult liver results in bile canalicular abnormalities
(A) Scanning electron micrograph, showing bile canalicular morphology from a wild-type mouse liver. (B) Scanning electron micrograph image from a β -catenin knockout mouse liver showing changes in bile canalicular morphology. Knockout mice have dilated, tortuous bile canaliculi and paucity of canalicular microvilli. Scale bar = 1 μ m.