Published in final edited form as:

Int J Biochem Cell Biol. 2011 July ; 43(7): 1021–1029. doi:10.1016/j.biocel.2009.09.001.

Role of Wnt/Beta-Catenin Signaling in Liver Metabolism and Cancer

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Abstract

Wnt/ β -catenin signaling is known for its role in embryogenesis as well as carcinogenesis. In the liver, it plays many critical roles during hepatic development and regeneration, and its dysregulation is evident in aberrant hepatic growth during various liver tumors. Its chief cellular roles in the liver include regulation of processes of cell proliferation, apoptosis, oxidative stress and differentiation, which in turn contributes to hepatic growth, zonation, xenobiotic metabolism and other metabolic processes inherent to the liver. Most of these functions of the Wnt/ β -catenin signaling are dictated through the highly temporal and tissue-specific or non-specific transcriptional targets of the pathway. In addition, some of the critical functions such as cell-cell adhesion and perhaps maintenance of various junctions that are critical from an epithelial cell biology perspective are also a function of β -catenin, which is the central component of the canonical Wnt pathway. Various animal models and clinical studies have demonstrated the spectra of Wnt/ β -catenin signaling in liver health and disease. Thus therapeutic modulation of this pathway for improved hepatic health is inevitable in the future. The current review discusses the advances in our understanding of the Wnt/ β -catenin signaling in liver physiology and pathology especially in hepatic metabolism and various tumors in adult liver and goes on to extrapolate the preclinical significance and possible translational implications of such findings.

1. The Beta-Catenin Signaling Pathway

The binding of an extracellular secreted glycoprotein Wnt to its cell surface receptor Fz transduces specific downstream, events commonly referred to as canonical or Wnt/ β -catenin signaling (Figure 1). Any of the Wnts (19 members in humans) in the absence of their negative regulators, bind to the seven-transmembrane receptor Frizzled (Fz) to form a ternary complex formation with LRP5 or LRP6 (or arrow) (Bhanot et al., 1996; Pinson et al., 2000). This complex is crucial in dictating the downstream canonical Wnt/ β -catenin signaling. Upon formation of the ternary complex, signal is transduced through multiple intermediate proteins to finally induce hypophosphorylation of β -catenin at the APC-axin-GSK3 β -CK complex. One such interaction is the activation of Dishevelled that blocks β -catenin degradation by various means (Li et al., 1999; Strovel et al., 2000; Sun et al., 2001). The end result is the hypophosphorylation of β -catenin at specific serine and threonine residues, its release from the multi-protein complex, cytoplasmic stabilization of its

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monomeric form and ensuing nuclear translocation where it binds to an HMG box containing DNA-binding protein TCF/LEF family member (Brannon et al., 1997; Riese et al., 1997).

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In a normal steady state where β -catenin activation is not necessary or in the absence of a Wnt signal or presence of Wnt inhibitors that sequester Wnt proteins, the free monomeric form of β -catenin in the cytoplasm is actively targeted for degradation by ubiquitination (Reviewed in (Peifer and Polakis, 2000)). In this situation, β -catenin is phosphorylated at serine and threonine residues in its amino terminal region, specifically at serine-45 (Ser45), Ser33, Ser33 and threonine-41 (Thr41) by Casein kinase I α (CKI α) and GSK3 (Amit et al., 2002; Behrens et al., 1998). CK and GSK3 β are part of a larger multiprotein degradation complex that includes axin and APC, which play a role in β -catenin degradation. Once phosphorylated this larger complex enables recognition and ubiquitination of β -catenin by β TrCP and its ensuing proteosomal degradation (Aberle et al., 1997). Thus free levels of β -catenin are kept low and it is prevented from translocating to the nucleus to induce target gene transcription. Transcription under the control of β -catenin/TCF4 is a highly complicated event and is reviewed comprehensively elsewhere (Stadeli et al., 2006). Briefly, in the absence of an activating Wnt signal, TCF inside the nucleus acts as a repressor of the target genes, but in the presence of a Wnt signal, β -catenin can induce the transcriptional activation capability of TCF. Once the TCF- β -catenin complex is formed in the nucleus, there is transcriptional activation of several target genes that have now been identified. An emerging concept is the stage and tissue-specificity of the transcriptional targets of this pathway. It is interesting to note that several Wnt components such as AXIN, DKK, dFz7, Fz2, FRP2, WISP, β TrCP and TCF are themselves targets, suggesting existence of several regulatory loops within this pathway.

2. Role of Beta-Catenin in Liver Growth, Metabolism and Homeostasis

β -Catenin signaling is essential for optimum hepatic homeostasis. Various studies have noted its expression during normal liver development during prenatal and postnatal stages. How β -catenin signaling is regulated in the normal liver remains unknown. To answer this question, it is relevant to identify normal expression and regulation of various components of this pathway in the liver. A recent study identified expression of many Wnt and Fz genes in adult mouse liver in all cell types (Zeng et al., 2007b). This demonstrates the extent of regulatory networks that exist to mediate β -catenin-dependent as well as β -catenin-independent effects of the Wnt signaling pathway. Additional studies are critical to address the specific roles of any of the various Wnt and Fz proteins normally expressed in the liver in various cell types in hepatic physiology and pathology.

2.1. Role of β -catenin signaling in prenatal liver development

The role of β -catenin during prenatal hepatic development is now better understood. β -Catenin is seen at membrane and in the cytoplasm and nucleus during early stages of hepatic development in mice (Micsenyi et al., 2004). This coincides with hepatoblast and immature proliferation. At later stages, there is a decrease in total β -catenin, although a smaller truncated species (~70kDa) persists and β -catenin is observed predominantly at the hepatocyte membrane with a few hepatocytes showing nuclear β -catenin as well. This pattern of β -catenin protein expression coincides with later hepatic development, a phase that is comprised of hepatocyte maturation, when immature hepatocytes begin to display cuboidal morphology, glycogen accumulation and acquisition of high expressions of genes responsible for hepatocyte function such as synthetic and xenobiotic genes (Monga et al., 2005). Many in vitro-studies have shown aberrations in hepatoblast and immature hepatocyte proliferation and hepatocyte maturation. A recent Foxa3-Cre driven β -catenin conditional null deletion, reiterates the above observations and while cell proliferation can be directly addressed as a function of β -catenin/TCF transactivation of genes such as *CCND1*, whether β -catenin is temporally regulating hepatocyte maturation via direct or indirect mechanisms remains under investigation (Tan et al., 2008). Similarly, β -catenin's role in biliary differentiation is evident in in-vitro and in-vivo studies, although the molecular basis remains undefined (Decaens et al., 2008; Hussain et al., 2004; Monga et al., 2003).

2.2. Role of β -catenin in postnatal hepatic growth

The liver continues to grow during neonatal stages. In fact in mice an early postnatal hepatic growth spurt is known for quite some time. Wnt/ β -catenin signaling was recently identified to be active during these stages and correlated well with ongoing hepatocyte proliferation (Apte et al., 2007). Several additional models have been employed that demonstrate a positive role of β -catenin in liver growth. A transgenic mice overexpressing a stable mutant of β -catenin generated under the transcriptional control of calbindin-D9K promoter and liver-specific enhancer of the *aldolase B* gene displayed 3-4 times larger livers due to increased cell proliferation (Cadoret et al., 2001). Interestingly they did not detect any changes in any of the conventional target genes of the pathway such as c-myc and cyclin-D1. Subsequent analysis of transgenic livers and subtractive hybridization led to identification genes involved in glutamine metabolism as targets of the Wnt/ β -catenin pathway. However, no signs of neoplastic transformation were reported in these animals, although APC-conditional null mice that exhibits β -catenin stabilization leads to development of robust hepatocellular cancer (Colnot et al., 2004). However, one has to take into account that many roles of APC other than negative regulation of β -catenin such as in cell polarity, cytokinesis and as a transcription factor are observed. Other transgenic mice have shown similar hepatic growth advantage (Harada et al., 2002; Tan et al., 2005) and also enabled identification of new targets such as EGFR (Tan et al., 2005).

2.3. Role of β -catenin in adult liver homeostasis

A useful approach to study the role of β -catenin in biology has been the generation and characterization of β -catenin conditional null mice, which have now been used extensively to study canonical Wnt signaling (Brault et al., 2001; Grigoryan et al., 2008). After the advent of floxed β -catenin mice, at least two independent groups including ours have utilized these mice for studying the role of Wnt/ β -catenin signaling in liver growth and metabolism (Sekine et al., 2007; Sekine et al., 2006; Tan et al., 2006). β -Catenin conditional null mice have identified a decreased liver weight/body weight ratio due to compromise chiefly in proliferation, along with mild effects on hepatocyte survival. This was secondary to decreased cyclin-D1, which is necessary for G1 to S phase transition and hence cell proliferation. Cyclin-D1 as a target in the liver is unquestionable and it is the chief mediator

of cell proliferation brought about by the Wnt/ β -catenin signaling as has been shown during hepatic development and liver regeneration (Sekine et al., 2007; Tan et al., 2006; Tan et al., 2008) (Figure 2). The modest phenotype evident in the hepatocyte-specific β -catenin knockout mice also suggests that there are redundant mechanisms are able to compensate for some of its functions including intercellular adhesion. Similarly, while these mice have no spontaneous phenotype, unpublished studies in our laboratory show an increased susceptibility to various forms of hepatic injury allowing to reiterate the relevance of normal β -catenin signaling in protein, fat and xenobiotic metabolism.

2.4. Role of β -Catenin in liver metabolism

The conditional knockout model has enabled verification of the regulatory role of β -catenin in ammonia metabolism as well as also reaffirmed its role in xenobiotic metabolism through regulation of various cytochrome P450s and glutathione s-transferases. In fact, three genes involved in glutamine metabolism encoding GS, ornithine aminotransferase, and the glutamate transporter-1 are all targets of Wnt/ β -catenin signaling in transgenic and knockout mice (Cadoret et al., 2002; Sekine et al., 2006; Tan et al., 2006). Likewise, overexpression of GS was noted selectively in mouse liver tumors that contained β -catenin mutations (Loeppen et al., 2002). Also several cytochrome P450 isoenzymes are upregulated in liver tumors harboring β -catenin mutations (Loeppen et al., 2005). Likewise, hepatocyte specific knockout of β -catenin in mice leads to a loss of expression of several P450s especially CYP2E1 and CYP1A2 (Sekine et al., 2006; Tan et al., 2006). More recently, it was also noted that β -catenin not only regulates the basal expression of drug-metabolizing enzymes but also determines the extent of relevant xenobiotic response to various inducers as well (Braeuning et al., 2009). In this report they showed that loss of β -catenin in a conditional animal led to not only basal alterations in expression of most drug metabolism-related genes, but also resulted in diminished responses to agonists of constitutive androstane receptor, aryl hydrocarbon receptor, and nuclear factor erythroid-related factor 2-dependent transcription with profound effect on phenotypes.

The above findings have also been complemented by the overall concept of APC/ β -catenin playing a major role in the zonation within the liver. While it has long been known that the liver is structurally divided into different zones, which express specific proteins and partake in differing metabolic functions, not much is known about its molecular basis.

Benhamouche et al. recently reported that β -catenin and APC distribution within various hepatic zones might be critical in the zonation process (Benhamouche et al., 2006). They identified high APC expression in periportal region with no β -catenin activation and conversely, identified absence of APC expression in centrizonal area where β -catenin activation is high, which maintains high expression of target genes such as GS and CYP2E1 (Figure 2). A more recent study reproduced the findings and expanded the expression of nuclear beta-catenin and glutamine synthetase in all zones of hepatic lobule after conditional loss of APC gene (Burke et al., 2009). This was accompanied by a decrease in the expression of carbamoylphosphate synthetase I. *Au contraire*, deletion of β -catenin led to a loss of GS and a concomitant increase of carbamoylphosphate synthetase I expression in absence or presence of APC. The authors also went on to show that c-Myc did not perturb the zonation pattern.

3. Beta-Catenin Signaling in Liver Tumors

Primary liver tumors presenting as hepatic masses are classified broadly as benign or malignant. The common benign tumors in the liver in which β -catenin signaling aberrations have been reported include the FNH and or the hepatic adenoma (HA). Malignant tumors are usually classified based on the lineage of transformed cells and various clinico-pathological characteristics. Broadly, the tumors originating from hepatic progenitors or

hepatoblasts are referred to as hepatoblastoma (HB) and occur in pediatric population. The tumors originating from more mature hepatocytes are referred to as hepatocellular cancer (HCC). While activation of β -catenin signaling is reported in both HB and HCC, the mechanisms and implications of β -catenin signaling will be discussed in HCC in this review.

3.1. Focal Nodular Hyperplasia (FNH)

These tumors are benign hepatic tumors next only to hemangiomas in incidence and exhibit a greater predisposition in the females. These tumors usually occur between the ages of 20-50 (Rebouissou et al., 2008a). The tumor is usually thought to be a hyperplastic response to either a pre-existing developmental arterial malformation or to increased blood flow, which leads to cellular hyperplasia. The molecular basis of FNH remains largely obscure, however, it is clear that FNH is polyclonal in nature in a significant subset of cases with only a minor subset composed of monoclonal tumor cells. Recent transcriptome analysis of FNH identified an activation of Wnt/ β -catenin pathway, although without any mutations in *CTNNB1* or the β -catenin gene (Rebouissou et al., 2008b). More recently, immunostaining for GS, a target of Wnt/ β -catenin signaling in the liver, has been proposed to identify FNH (Bioulac-Sage et al., 2008). While the significance of these findings is unclear, especially since these tumors are thought to be a result of vascular disturbances, these observations might represent an alternate mechanism of β -catenin activation such as growth factor-dependent activation (Chen et al., 2002; Monga et al., 2002).

3.2. Hepatic adenoma

Hepatic adenomas (HA) are benign liver tumors that occur in greater frequency in females and are observed as benign proliferation of hepatocytes in an otherwise normal liver (Rebouissou et al., 2008a). HAs have been associated with the use of estrogen-containing oral contraceptives or androgen-containing steroid anabolic drugs. Additionally, glycogen storage diseases, especially Type I (von Gierke) and Type III are also known risk factors for the development of hepatic adenoma. Macroscopically, HA present as solitary, yellowish masses due to lipid accumulation and gives a pseudoencapsulated appearance because of the compression of adjacent hepatic tissue. Microscopically, HA's may show areas of fatty deposits and hemorrhage, cord- or plate-like arrangements of larger hepatocytes containing excessive glycogen and fat, sinusoidal dilatation owing to the effects of arterial pressure as these tumors lack a portal venous supply, absent bile ductules, and presence of few and non-functioning Kupffer cells. The extensive hypervascularity and lack of a true capsule makes this tumor prone to hemorrhage. The tumors can regress following discontinuation of inciting factors such as oral contraceptives; at other times they continue to exhibit a stable or progressive disease. However, the risk of associated hemorrhage and neoplastic transformation is always present. In view of these risks, surgical resection is often recommended. Performance of surgery, however, is influenced by the multiplicity or size of the tumors and associated symptoms on one hand and surgical risk to the patient on the other.

Thus molecular basis of the tumors is better understood (Rebouissou et al., 2008a; Zucman-Rossi et al., 2006). A high percentage of hepatic adenomas display inactivating mutations in the HNF1 α gene. It should be noted that HNF1 α knockout mice develop fatty liver and hepatocyte dysplasia supporting a clear role of this protein in lipid metabolism (Lee et al., 1998; Shih et al., 2001). Biallelic inactivating mutations in HNF1 α or Tcf1 gene (not the β -catenin binding factor T-cell factor 1 or TCF1 protein encoded by *Tcf7*) gene were identified in 35-50% of HA (Bluteau et al., 2002). The tumors in this scenario display marked steatosis and excess glycogen accumulation. These observations were explained by the role of HNF1 α in regulating liver fatty acid-binding protein, which plays a role in fatty acid trafficking in hepatocytes, and glucose-6-phosphate increase, respectively (Rebouissou et

al., 2007). HA with HNF1 α inactivation display an extremely low risk of malignant transformation.

In another subset of HA's, Wnt/ β -catenin activation is observed secondary to mutations in *CTNNB1* gene that eventually effect the degradation of β -catenin protein. These adenomas can range from 15-46%, but the numbers might be closer to the lower percentage when only CTNNB1 mutations as a mechanism of β -catenin activation are considered (Torbenson et al., 2002; Zucman-Rossi et al., 2006). These tumors show frequent cytological abnormalities and pseudoglandular formation and have been shown to occur at abnormally higher frequency in males. Most importantly, these HAs have been shown to possess a higher propensity for malignant transformation.

There is also yet another group of HA's that display acute phase inflammation, histologically (Rebouissou et al., 2008a). In a recent study the molecular basis of these HA's was unveiled (Rebouissou et al., 2009). These forms of adenomas are seen in patients with high body mass index and excessive alcohol intake. Such tumors show sinusoidal dilatation, inflammatory infiltrates and vessel dystrophy. Most characteristic feature of these tumors is the presence of inflammatory infiltrates along with an increase in the expression of inflammatory proteins in tumorous hepatocytes. This was attributed to an enhanced IL-6 signaling that occurred due to somatic gain-of-function mutations in the *IL6ST* gene, which encodes gp130 (IL-6 co-receptor) in around 60% of inflammatory Has. This leads to the activation of signal transducer and activator of transcription 3 (STAT3) even in the absence of ligand. Interestingly, the analysis of HCC also revealed the rare gp130 mutations were concordant with activating mutations in *CTNNB1*, suggesting cooperation of these two independent pathways in a subset of these tumors especially in their malignant transformation.

3.3. Hepatocellular cancer

HCC is the most common primary tumor of the liver accounting for 85% of all primary malignant tumors. It is the fifth commonest malignancy worldwide and third most-common cause of death related to cancers (El-Serag, 2004; Parkin et al., 2005). Most HCCs occur in the setting of chronic liver injury and the risk factors include hepatitis, chronic alcohol abuse, aflatoxins ingestion, NASH, biliary injury (primary biliary cirrhosis and primary sclerosing cholangitis) and other metabolic liver diseases. HCC used to be a common malignancy in underdeveloped or developing countries but is now on the rise in developed countries (Bergsland and Venook, 2000; McKillop et al., 2006). The increasing incidence of HCC in developed countries is attributed to increasing incidence of hepatitis C and NASH. HCC afflicts three times more men than women and overall incidence increases with age, especially in western world, although trends are now changing. In fact peak incidence of HCC in a recent study was between the ages of 45-60 years (El-Serag, 2004).

Much of the HCC occurs in the background of cirrhosis. Several chronic hepatic pathologies lead to development of fibrosis and cirrhosis. In the setting of cirrhosis, liver function is ascertained by the presence of regenerating nodules, which are a function of the regenerative capacity of surviving hepatocytes (Friedman, 2008). However, some of these nodules evolve into low- and high-grade dysplastic nodules, which then lead to HCC (Villanueva et al., 2007). These nodules are a result of genomic instability over time ranging from chromosomal losses, gains, rearrangements, somatic mutations in genes (some induced directly others adaptive), and may be due to the presence of metabolites, reactive oxygen species, nutritional stress or other forms of inciting products as result of chronic hepatic injury and local microenvironment and milieu. While not necessary, the cell proliferation within a regenerating nodule makes the hepatocytes more amenable to the genetic alterations and eventually transformation. Once transformed, the tumor cell clearly is still in the milieu

of regenerative environment and the same growth factors and cytokines that promoted regeneration, now promote growth and proliferation of the transformed cells. This hypothesis will need to be strengthened further in a significant subset of HCCs although much of the circumstantial evidence exists supporting the Jekyll and Hyde disposition of regeneration in the process of HCC development. One must also be reminded that while presence of cirrhosis is typical, more recently, there have been observations in larger data sets of patients, where HCC was also evident in the presence of fibrosis but absence of cirrhosis (Lok et al., 2009). As discussed previously, a subset of hepatic adenoma also proceed to evolve into HCC.

The possibility of a cancer stem cell origin of some of the HCC is also being entertained, although it remains highly controversial. Cancer stem cells are formed by mutations in the normal existing stem cells or a progenitor cell within a tissue. It has also been suggested that perfectly mature cells such as hepatocytes can dedifferentiate in progenitor-like cells and could also be the targets of mutations (Sell and Leffert, 2008). Dysplastic lesions within liver have been shown to have pre-neoplastic precursors. In addition, a subset of HCCs exhibit mixed hepatocholangiocarcinoma phenotype that might represent bipotential stem cell origin of these tumors (Tsuneyama et al., 2003). In addition, some early HCCs have been shown to harbor smaller proliferating oval cells or stem cells. In fact the pathways that are known to regulate stem cell renewal such as the Wnt, Hedgehog and Notch have been shown to play a role in oval cell activation as well as in a subset of HCCs (Sell and Leffert, 2008). The positive impact of Wnt/ β -catenin signaling on rat and mouse oval cells was recently reported in two independent studies (Apte et al., 2008). However, whether these characteristics are indicative of cancer stem cells or are merely 'normal' tumor cells of the hepatocellular cancer, which possess the features of hepatoblasts or fetal hepatocyte and have the potential of clonal growth, remains to be further investigated.

A disease of extremely poor prognosis, HCC remains among the leading causes of mortality and morbidity around the globe. The disease bears a strong etiological association with viral hepatitis, hemochromatosis, chemical carcinogens, and toxins (mycotoxins) (Reviewed in (Tannapfel and Wittekind, 2002)). Preexisting cirrhosis due to any number of factors such as metabolic disease, toxins, inflammation, infection, biliary or cryptogenic predisposes to HCC.

Wnt/ β -catenin activation has been implicated in many cancers and is one of the important aberrant pathway identified in HCC in animal and patients (Laurent-Puig et al., 2001; Peifer and Polakis, 2000; Polakis, 2000). Abnormal localization of cadherins and catenins in liver cancer was first shown by immunohistochemistry (Ihara et al., 1996). A more comprehensive study identified anomalous β -catenin expression as well as mutations in the *CTNNB1* in around 25% of all HCC cases and up to 50% of all hepatic tumors in transgenic lines such as c-myc or H-ras (de La Coste et al., 1998). Several subsequent studies corroborated these observations although the mutations in β -catenin gene ranged from 12%-34%, whereas abnormal β -catenin redistribution has been reported in up to 90% of HCCs suggesting additional mechanisms (Kondo et al., 1999; Legoix et al., 1999; Nhieu et al., 1999; Wong et al., 2001). A recent study in 32 patients also identified activating mutations in *CTNNB1* in around 28% of patients (Cieply et al., 2009). Table 1 shows common mutations in *CTNNB1* in HCC patients.

Additional mechanisms of β -catenin activation have also been described. AXIN1 and AXIN2 mutations were also detected in around 5%-10% and 3% of HCCs respectively (Sato et al., 2000; Taniguchi et al., 2002) (summarized in Table 2). Reports analyzing GSK3 β studies are conflicting although elevated levels of inactive GSK3 β are observed in HCCs harboring β -catenin accumulation (Ban et al., 2003; Cui et al., 2003; Gotoh et al.,

2003). Activation of Frizzled-7/Wnt-3a axis has been reported in significant numbers of HCCs analyzed (Kim et al., 2008; Merle et al., 2004). Epigenetic inactivation of inhibitors of Wnt signaling such as soluble FRP has also been reported in HCC (Takagi et al., 2008). FL-HCC showed significant tyrosine-phosphorylated β -catenin suggesting receptor tyrosine kinase activation in these tumors (Cieply et al., 2009). Recently increased EGFR protein expression has also been identified in significant numbers of FL-HCC and this increase appeared to be secondary to gains of chromosome 7 (Buckley et al., 2006). Thus EGFR antagonists and other receptor tyrosine kinase inhibitors might have a useful role in treatment of FL-HCC. There have been reports identifying differences in molecular targets of the pathway secondary to modes of β -catenin activation (Zucman-Rossi et al., 2007).

β -Catenin activation in HCC is often observed as nuclear and/or cytoplasmic with or without membranous localization (Figure 3). While the exact basis of such differences is unknown, it could represent heterogeneity in mechanisms of β -catenin activation. In addition, what is true activation of β -catenin pathway especially in clinical specimen is unclear. Is it sufficient to label the presence of cytoplasmic and/or nuclear β -catenin in the presence or absence of membranous localization as indicators of β -catenin activation? Can β -catenin activation be sufficiently identified on the basis of induction of target proteins such as GS or Axin-2? Is the presence of increased cyclin-D1 in the presence of β -catenin stabilization specific enough to implicate Wnt signaling in proliferation in that state? Finally, a major caveat is that many targets of β -catenin signaling are feed back inhibitors of the pathway and thus regulate the amplitude of Wnt signaling. How does the expression of such targets impact β -catenin activation in various tumor subsets and how that would impact the overall tumor behavior remains unexplored. There might be additional geographical, dietary and etiological factors, which might dictate the predilection of Wnt/ β -catenin signaling in the pathogenesis of HCC. One study detected an inverse correlation between *β -catenin* mutations and loss of heterozygosity in the genome suggesting chromosomal instability (involving tumor suppressor genes) and mutations in *CTNNB1* representing alternative modes of tumor progression (Legoix et al., 1999). Interestingly a much higher frequency of *CTNNB1* mutations are observed in HCC associated with HCV infection. More than 40% of HCV associated HCCs demonstrate stabilizing mutations in *β -catenin* gene (mostly at Ser45) as well as nuclear accumulation of its protein (Huang et al., 1999). HBV related HCC had overall lesser frequent *β -catenin* mutations (Hsu et al., 2000). Also, although mutations in its gene were infrequent, aflatoxin associated HCC showed increased accumulation of β -catenin in around 45% of tumors (Devereux et al., 2001). Analysis has also extended to identify distinct molecular signatures of HCC arising in cirrhotic versus non-cirrhotic livers and although preliminary, this analysis suggests unique pathogenetic events in the two subsets. While HCC in non-cirrhotic livers demonstrates more frequent Wnt/ β -catenin involvement along with other pathways, HCC arising in cirrhosis showed mainly p53 alterations (Tannapfel and Wittekind, 2002). Very interestingly, our recent analysis of HCC also showed a lack of overt cirrhosis or bridging fibrosis in around 60-70% of all β -catenin-mutated group of HCCs irrespective of etiology of the disease (Cieply et al., 2009). It was also interesting to note that in a recent analysis in HALT-C trial, a significant subset of patients with Hepatitis C (17%) who developed HCC lacked overt cirrhosis (Lok et al., 2009). While no mechanism of hepatocarcinogenesis in these patients is known, β -catenin mutations might play an important independent risk factor in the development of HCC in the absence of underlying cirrhosis. Finally, another study reported higher frequency of Wnt/ β -catenin aberrations in HCV associated HCCs as compared to alcoholism associated HCC that more frequently involved RB1 and p53 pathways (Edamoto et al., 2003).

What is the impact of aberrant β -catenin activation on the biology of the disease in HCC? There is a lack of consensus as to whether β -catenin activation imparts a poor or better tumor behavior. There are examples of β -catenin activation associated with poorer prognosis

(Huang et al., 1999; Nhieu et al., 1999) and also with non-invasive form of tumor and better prognosis (Hsu et al., 2000; Mao et al., 2001). Additional reports have correlated overexpression of non-nuclear type β -catenin with poor cell differentiation, larger tumor size and significantly shorter disease-free survival lengths (Wong et al., 2001). Another study found a significant relationship of nuclear cellular retinol-binding protein-1, nuclear β -catenin, low Ki-67 positivity and favorable prognosis and 2-year survival (Schmitt-Graff et al., 2003). Greater tumor size was also demonstrated in β -catenin-mutated HCC as compared to β -catenin-non-mutated group in another study (Laurent-Puig et al., 2001). In our recent study, we identified presence of concomitant vascular invasion (mostly microvascular) and tumor size of ≥ 5 cm. This enabled us to classify the β -catenin-mutated tumors as more advanced as compared to the non-mutated groups, based on presence of concomitant invasion and greater tumor size (Vauthey et al., 2002). Thus, future prospective studies to determine the outcome of HCC as a function of β -catenin activation will be essential. Specifically such studies would need to take into account the geographical localization, etiology and heterogeneity in patients as well as the modes of β -catenin activation in the patients.

Wnt/ β -catenin pathway in HCC in animal models is also unsettling. The studies involving β -catenin transgenic mice that overexpress truncated *β -catenin* in liver do not show any evidence of spontaneous carcinogenesis (Cadoret et al., 2001). Another study overexpressing non-truncated human *β -catenin* gene under transcriptional control of albumin promoter/enhancer also yielded no tumors in the liver (Tan et al., 2005). Similar lack of tumorigenesis was also observed following adenoviral-mediated overexpression of dominant stable β -catenin mutant in liver (Harada et al., 2002). Interestingly APC-null mice showed significant HCC through activation of β -catenin (Colnot et al., 2004). Overall, it might be β -catenin mutations might be insufficient on their own and require cooperation with other pathways for hepatocarcinogenesis. While interesting, it is important to take into account the many β -catenin-independent roles of APC in biological processes including roles in directly regulating DNA replication via direct interaction with DNA and in cell polarity (Prosperi et al., 2009; Qian et al., 2008). Devising newer liver models including transgenic lines as well as newer chemical carcinogenesis strategies such as tumors induced by 2-amino-3, 4-dimethylimidazo [4,5-f] quinoline, that selectively induce anomalies in the Wnt/ β -catenin pathway would be really useful to better understand the role and regulation of this pathway as well as have significant therapeutic implications (Huang et al., 2003).

4. Future Prospects

4.1. Therapeutic implications of Wnt/ β -catenin signaling in Regenerative Medicine

The role of β -catenin in liver development and regeneration in regulating cellular events such as proliferation, survival, differentiation and maturation is relevant in the arena regenerative medicine. Modulation of Wnt/ β -catenin signaling for the purposes of hepatic tissue engineering, cell therapeutics and improving regeneration might have significant translational implications. Furthermore, as additional studies uncover the roles of Wnt/ β -catenin signaling in integrative metabolism of fat, protein and carbohydrate due to its direct or indirect effects, will also be of value to hepatic regenerative medicine.

The lack of livers for transplantation remains a serious problem for patients with chronic or acute on chronic liver diseases. Since the statistics are troubling, there are alternate strategies that clearly are of relevance and range from modalities that bridge the patients to transplant or may be therapeutic due to known or unknown mechanisms. The various modalities reflect the broad range of ongoing research in many laboratories around the globe. These areas of hepatic regenerative medicine include stem cell differentiation into hepatocytes, transplantation of hepatocyte or transdifferentiated stem cells and use of similar cell types in

artificial liver devices for supportive care. While none of these modalities are approved or widely used measures in the clinic with some exceptions, they are at different stages of development. Modulation of Wnt signaling will be clearly an important step in the multi-step process of proper stem cell differentiation to hepatocyte. In fact there are studies that now demonstrate successful acquisition of hepatocyte phenotype following modulation of Wnt/ β -catenin signaling in human ES cells, mesenchymal stem cells derived from bone or cord blood (Hay et al., 2008; Ishii et al., 2008; Ke et al., 2008; Yoshida et al., 2007). These studies are stemming from various published studies in liver development in mice, chick, zebrafish and xenopus that have identified a highly temporal regulation of β -catenin signaling during this complex process (reviewed in (Nejak-Bowen and Monga, 2008)). Growth factor usage for therapy in hepatic insufficiency has also pre-clinically validated in many models of acute liver failure. The identification of such factors has come through understanding of the complex process of liver regeneration in a widely accepted model of partial hepatectomy. Wnt/ β -catenin signaling has been shown to be relevant in this process and could be a usable target to stimulate regeneration. This has been recently shown in a zebrafish model and additional studies are on the horizon, although how to induce regulated β -catenin activation for therapy remains a valid question (Goessling et al., 2008). It should be pointed out that various growth factors are also known to activate β -catenin through a Wnt-independent manner and through tyrosine phosphorylation of β -catenin. Factors such as HGF and EGF among others are known to induce β -catenin activation (Bonvini et al., 2001; Monga et al., 2002). Thus it might be feasible to use this mechanism as a possible means of β -catenin activation for therapies directed at stimulating hepatic regeneration.

4.2. Therapeutic implications of β -catenin signaling in HCC

Due to aberrant activation of Wnt/ β -catenin pathway in multiple cancers including HCC, it is an acceptable therapeutic target (Llovet and Bruix, 2008). The most crucial component of this pathway especially in liver is perhaps β -catenin, which plays an array of functions as a transcriptional co-activator and a cell-cell adhesion protein. Several proof-of-principle preclinical studies have clearly demonstrated an important benefit of therapeutic inhibition of β -catenin as a means of treatment for HCC (Zeng et al., 2007a). A primary question is to determine if β -catenin inhibition in clinical scenario in the liver will be a feasible option owing to its critical roles in hepatic homeostasis. Some optimism comes from the preclinical models such as β -catenin conditional null mice, which lead to a viable animal with only a mild phenotype of 10-15% lower liver weight to body weight ratio (Sekine et al., 2007; Sekine et al., 2006; Tan et al., 2006). This suggests that there are redundant mechanisms that enable at least intercellular adhesion in the absence of β -catenin in the hepatocytes. Also, in an unchallenged state, β -catenin absence does not induce any dramatic hepatic pathology in these mice. Whether loss of β -catenin will be attainable in patients without explicit side effects especially in the liver or perhaps other tissues, remains unexplored. While liver is unique and derives its dual blood supply from portal vein (75%) and hepatic artery (25%), hepatoma is almost exclusively supplied by hepatic artery. While this is done at specific multidisciplinary centers, local delivery of a modulator of Wnt/ β -catenin pathway through such a modality might be a strategy to circumvent untoward side effects or achieve higher local concentrations of an agent.

Specific inhibitors of β -catenin signaling in preclinical applications are beginning to surface. Agents such as PKF115-584 (Novartis) and ICG-001 have demonstrated in vitro and in vivo inhibition of Wnt/ β -catenin signaling and are still in early phases of drug discovery and development (Eguchi et al., 2005; Minke et al., 2009; Sukhdeo et al., 2007). While various cox-2 inhibitors such as rofecoxib have shown efficacy in decreasing β -catenin levels along with shrinkage of tumors (Yao et al., 2003). R-Etodolac, an enantiomer of a cox-2 inhibitor that lacks an inhibitory effect on cox-2 has also shown anti- β -catenin effect (Behari et al.,

2007). This might be more meaningful in light of and the unwanted side effects associated with cox-2 inhibitors. Based on observations of increased receptor tyrosine kinase signaling in hepatocellular cancers especially in the subset of tumors such as fibrolamellar HCC and focal nodular hyperplasia, targeting tyrosine-phosphorylated β -catenin might be of relevance. Previously, Gleevec has shown to decrease tyrosine-phosphorylated β -catenin levels only (Zhou et al., 2003). Similarly, recently approved Sorafenib for HCC treatment might have more dramatic response in HCC showing receptor tyrosine kinase activation, which also leads to the tyrosine phosphorylation of β -catenin (Llovet et al., 2008). Another group of agents including Exisulind and analogues that are inhibitors of cyclic GMP phosphodiesterases have been shown to activate protein kinase G that in turn decrease β -catenin levels via a novel GSK3 β -independent processing mechanism (Li et al., 2002). Finally, various studies have identified novel gene targets of β -catenin in liver health and disease. For cancer perspective, it will be especially relevant to therapeutically target downstream transcriptional targets of β -catenin/TCF4 complex, which are directly involved in regulating cell survival and proliferation. Cyclin-D1 and EGFR in liver and survivin, c-myc and others in other tissues are clear examples of such downstream targets that could have implications in oncology.

Similarly, as Wnt/ β -catenin signaling is also at the heart of hepatic xenobiotic and ammonia metabolism, it might be feasible to take advantages to selectively target tumors in the liver based on metabolic profiles of the tumor. As we are aware the overexpression of certain P450s, GS and other yet unidentified targets impart a specific metabolic phenotype to β -catenin active tumors, which could have therapeutic potential. Thus while the modes of therapeutic inhibition of Wnt/ β -catenin signaling are many although preliminary at this stage its successful inhibition would be one key therapeutic strategy in treatment or chemoprophylaxis of various hepatic tumors (Thompson and Monga, 2007).

Acknowledgments

Grant Support: This study was funded by NIH grants 1R01DK62277 and 1R01CA124414 to SPSM and by Rango's Fund for the Enhancement of Pathology Research.

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Abbreviations

LRP	Density Lipoprotein (LDL) related protein
GSK	glycogen synthase kinase
APC	adenomatous polyposis coli
CK	casein kinase
TCF	T cell factor
LEF	Lymphoid enhancing factor
βTrCPβ	transducin repeat-containing protein
DKK	dickkopf
FRP	frizzled related peptide
Fz	frizzled
WISP	Wnt1 inducible signaling pathway protein
EGFR	epidermal growth factor receptor
GS	glutamine synthetase
FNH	focal nodular hyperplasia
HA	hepatic adenoma
HB	hepatoblastoma
HCC	hepatocellular cancer
HNF	hepatocyte nuclear factor
NASH	non-alcoholic steatohepatitis
FL	HCC-fibrolamellar hepatocellular cancer

HBV	hepatitis B virus
HCV	hepatitis C virus
ES cell	embryonic stem cell
cox	cyclooxygenase

Figure 1.
A simplified schematic of Wnt/beta-catenin signaling.

Figure 2.

Beta-catenin target genes in the liver: role in cell proliferation and metabolism.

- A.** Loss of beta-catenin in the liver (conditional knockout or KO) leads to decreased gene expression of cyclin-D1 and numbers of hepatocytes in S-phase in as shown by immunohistochemistry for Ki-67, as compared to littermate controls (Con).
- B.** Loss of beta-catenin leads to loss of GS in KO, whereas a single layer of hepatocytes (arrowhead) around central vein is positive in Con liver.

Figure 3.

Stabilization and nuclear translocation of β -catenin is observed in HCC.

- A.** Normal livers display β -catenin predominantly at the hepatocyte membrane.
- B.** An increase in total β -catenin and its nuclear translocation (arrowhead) is observed in a representative HCC liver sample.

Table 1
Common mutations in β -catenin gene (CTNNB1) in HCC patients

Study	Cases with mutations	Mutation sites					Additional information
		S33	S37	S45	T41	OTHERS (includ. Del ^{ac})	
(Cieply et al., 2009)	9/32	2	2	1	2	2	2 patients with mutations at D32
(Cui et al., 2003)	15/45				5	10	No GSK3 β mutations
(Yamamoto et al., 2003)	16/38	2	2	3	1	10	Multiple mutations in 2 patients
(Taniguchi et al., 2002)	14/73	1		7	1	5	1 insertion between S33 and G34
(Devereux et al., 2001)	5/62	1			2	2	Aflatoxin study
(Huang et al., 1999)	9/22	1	3	2	1	3	Multiple mutations in 1 patient
(Nhieu et al., 1999)	12/35	1		2	2	7	Multiple mutations in 2 patients
(Legoix et al., 1999)	21/119	3	2	8	4	5	Multiple mutations in a patient
(de La Coste et al., 1998)	6/26	2	1	1		2	1 patient showed deletion
(de La Coste et al., 1998)	9/32	2	2	1	2	2	2 patients with mutations at D32

Table 2
Common mechanisms of activation of Wnt/ β -catenin signaling in HCC

1	Mutations in β -catenin gene (<i>CTNNB1</i>)
2	Mutations in <i>AXIN1</i> gene
3	Frizzled-7 upregulation
4	HBx/URG11 expression in Hepatitis B
5	Methylation of sFRP1, sFRP2 and sFRP5
6	Upregulation of Frizzled-3/6/7
7	Upregulation of Wnt3/4/5a
8	Tyrosine-phosphorylation dependent β -catenin activation
9	Others/unknown