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Review

Wnt/β-catenin signaling in hepatic organogenesis

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Wnt/β-catenin signaling has come to the forefront of liver biology in recent years. This pathway regulates key pathophysiological events inherent to the liver including development, regeneration and cancer, by dictating several biological processes such as proliferation, apoptosis, differentiation, adhesion, zonation and metabolism in various cells of the liver. This review will examine the studies that have uncovered the relevant roles of Wnt/β-catenin signaling during the process of liver development. We will discuss the potential roles of Wnt/β-catenin signaling during the phases of development, including competence, hepatic induction, expansion and morphogenesis. In addition, we will discuss the role of negative and positive regulation of this pathway and how the temporal expression of Wnt/β-catenin can direct key processes during hepatic development. We will also identify some of the major deficits in the current understanding of the role of Wnt/β-catenin signaling in liver development in order to provide a perspective for future studies. Thus, this review will provide a contextual overview of the role of Wnt/β-catenin signaling during hepatic organogenesis.

The Wnt/β-catenin pathway is an evolutionarily well-conserved pathway that has proven to be essential to normal cellular processes such as development, growth, survival, regeneration and self-renewal.[1-5] Its diverse functions also include the initiation and progression of cancer.[6] In fact, one area in which this pathway has been extensively studied is in liver cancer.

Mutations of Wnt/β-catenin pathway members in hepatocarcinogenesis are common. For example, 90–100% of hepatoblastomas contain mutations in adenomatous polyposis coli (APC), CTNNB1 and/or Axin1/2, which causes cytoplasmic and nuclear localization of β-catenin.[7-9] Axin1 and β-catenin mutations have also been identified in approximately 25% of hepatocellular carcinomas,[10-12] while overexpression of the frizzled-7 receptor[13] and glycogen synthase kinase-3 (GSK-3) inactivation[14] can also lead to aberrant β-catenin pathway activation. The dysregulation of this pathway in hepatic cancers makes it an attractive target for potential therapies, and experimental treatment in vivo has shown promising results. For example, inhibiting β-catenin expression by siRNA or R-Etodolac decreases proliferation and survival of human hepatoma cell lines.[15,16] Since cancer recapitulates development, determining the timing of β-catenin activation during hepatogenesis will help us to better understand the inappropriate activation of this pathway in hepatocarcinogenesis.

Recent work has elucidated the role of β-catenin signaling in the liver, and has highlighted its essential role in liver health and disease.[17] In addition, emerging evidence suggests that this pathway plays a key role in liver organogenesis.

The Wnt/β-Catenin Pathway

The Wnt/β-catenin pathway is inactive in normal unstimulated cells. In this steady-state condition, β-catenin, the central player in this signaling cascade, is bound in a complex with Axin, APC and GSK3. In the absence of Wnt, β-catenin is phosphorylated by casein kinase 1 (CK1) and GSK3α/β at serine/threonine residues located at the N-terminal region of the protein.[18] This phosphorylation targets β-catenin for ubiquitination and ultimate degradation by the proteasome. When Wnt proteins bind to the Frizzled receptor on the surface of cells, it activates the canonical Wnt pathway. The Wnt/Frizzled interaction induces association with the low-density lipoprotein receptor related protein (LRP) 5/6, and this complex then recruits Dishevelled, which is thought to inactivate GSKβ.[19] Inactivation of GSKβ leads to the absence of β-catenin phosphorylation, which subsequently releases it from the Axin/APC/GSK3 complex. β-catenin then translocates to the nucleus, where it binds to lymphoid enhancer-binding factor 1/T cell-specific transcription factor (LEF/TCF), displaces the transcriptional inhibitor Groucho, and in complex with TCF activates target genes important in proliferation and differentiation.[3]

In addition to the above-mentioned participants involved in the canonical Wnt signaling pathway, several other proteins are known to interact with β-catenin. E-cadherin, along with α-catenin, forms a complex with β-catenin at the surface of hepatocytes.[20] α-catenin binds to actin, anchoring the complex to the cytoskeleton. The β-catenin/E-cadherin interaction also mediates cell-cell adhesion and is regulated by the phosphorylation of β-catenin at a specific tyrosine residue (Y654).[21] Specifically in liver, this causes dissociation of the complex and subsequent degradation of E-cadherin, resulting in a loss of adherens junctions and impaired apical trafficking in hepatocytes.[22] Loss of adhesion may also contribute to motility, which is an important component of the cellular response in processes such as development, regeneration and cancer growth.

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TGFβ, a known regulator of self-renewal which has also been implicated in the modulation of hepatocellular carcinoma,[23] plays an important role in E-cadherin/β-catenin interactions as well. TGFβ-mediated loss of E-cadherin results in the release of β-catenin from cell-cell contacts and its subsequent translocation to the cytoplasm. Active β-catenin thus leads to the increased cell motility and invasive phenotype seen in gastrointestinal and liver cancers.[24,25]

Another important interaction involving β-catenin in hepatocytes is the β-catenin/Met complex. Met is the receptor for hepatocyte growth factor (HGF), a known mitogen, motogen and morphogen for the liver.[26] We have previously shown that Met and β-catenin associate at the surface of hepatocytes; binding of HGF to its receptor induces phosphorylated Met to phosphorylate β-catenin, which results in its translocation to the nucleus.[27] Another study by our lab found that injecting the human HGF gene into mice leads to hepatomegaly via dissociation of the Met/β-catenin complex and induction of the β-catenin pathway.[28] β-catenin also regulates HGF-induced cell morphogenesis,[29] and interactions between Met and a mutated active form of β-catenin have been found to facilitate hepatocellular carcinoma.[30] The Met/β-catenin pathways thus cooperate to induce hepatocyte proliferation both in vitro and in vivo.

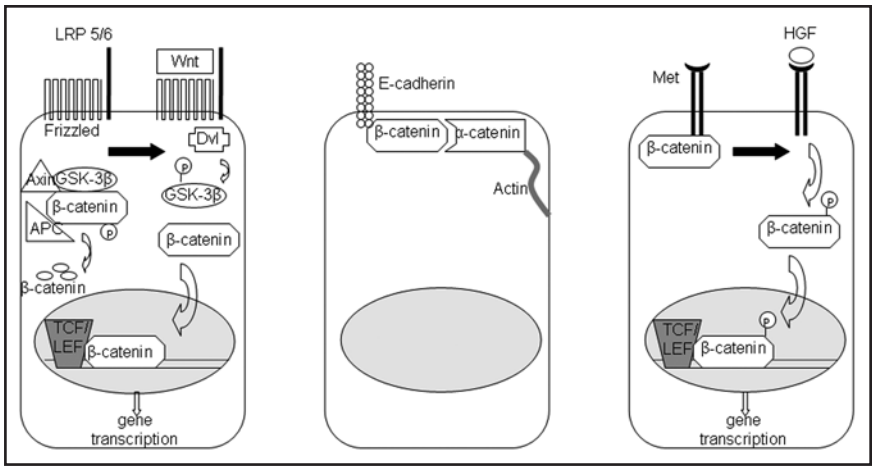

Figure 1. The three major roles of β-catenin in liver physiology. Left: in the presence of Wnt, β-catenin is released from its inactivating complex and translocates to the nucleus, where it activates genes essential for proliferation, growth and regeneration of the liver. Middle: β-catenin mediates cell-cell adhesion through its interaction with e-cadherin on the hepatocyte membrane. Right: in the presence of HGF, β-catenin, which associates with Met at the surface of hepatocytes, is phosphorylated and translocates to the nucleus to turn on genes important in proliferation and morphogenesis.

This review will discuss these pathways and interactions—Wnt/β-catenin, β-catenin/E-cadherin, TGFβ/β-catenin and Met/β-catenin—in the context of prenatal liver development (Fig. 1). A general discussion of the role of β-catenin in normal liver growth and regeneration, as well as a general overview of embryonic liver development, will provide the background for this discussion.

Wnt/β-catenin Signaling in Normal Liver Growth and Regeneration

The Wnt/β-catenin pathway plays a key role in controlling postnatal liver growth. Our laboratory found an increase in β-catenin levels in wild-type mice shortly after birth,[31] which serves to promote hepatic growth during postnatal development. Indeed, increased β-catenin translocation to the nucleus correlates with an increase in cell proliferation between 5–20 postnatal days.[32] Further, mice expressing a conditional deletion of β-catenin generated by others and our laboratory showed a significant decrease in the liver weight/body weight ratio (15–25%) in mice older than two months.[33,34] This decrease is correlated with basal decrease in cellular proliferation, which is due to a deficient cyclin-D1 response.

Several studies have addressed the role of constitutively active β-catenin on postnatal growth. Mice expressing an oncogenic form of β-catenin demonstrated hyperplasia and resulting hepatomegaly as young as 3–4 weeks after birth.[35] Other reports have described hepatomegaly soon after birth in mouse strains where a dominant stable form of β-catenin was activated by adenoviral inoculation.[36] Finally, we have recently generated transgenic mice overexpressing wild-type β-catenin and observed a 15% increase in liver size in these mice compared to normal wild-type aged matched controls.[37] All three mouse models showed a correlation between increased nuclear localization of β-catenin and increased proliferation in the transgenic overexpressing mice, implicating activation of cell cycle regulators by β-catenin in the resulting phenotype. Notably, all of the β-catenin-overexpressing mouse models showed a distinct lack of spontaneous hepatic tumors, suggesting that β-catenin alone may be insufficient to cause tumorigenesis.

In adult resting liver, the Wnt/β-catenin pathway is quiescent. This steady-state condition is characterized by the phosphorylation and subsequent degradation that is the hallmark of β-catenin turnover; hence, β-catenin is localized at the cell membrane and is largely absent from the cytoplasm and nucleus.[38] Therefore, when liver is not being challenged by chemical, metabolic or dietary stress, β-catenin is not required for normal physiologic function.[34]

However, during liver regeneration, levels of β-catenin are dramatically increased. The most common method used to study liver regeneration is the partial hepatectomy (PHx) model, in which two-thirds of the rat or mouse liver is removed; the remaining lobes enlarge to recapitulate the original liver mass.[39] This model is an ideal environment to study the role of β-catenin in controlled growth after injury. In a rat model, an increase in β-catenin protein expression was seen as early as 1–5 minutes post-PHx;[40] this increase was not due to an increase in mRNA expression, but rather to a decrease in protein degradation, the result of a change in steady-state kinetics. This expression was transient, and β-catenin levels returned to normal at 48 hours. Translocation of β-catenin to the nucleus, starting at 5 minutes after PHx and continuing until 48 hours post-PHx, contributes to the increase in cyclin D1 and c-myc and the concomitant increase in cellular proliferation.[40]

The importance of β-catenin to liver regeneration is highlighted by three studies in which β-catenin is removed or absent from the liver. When a β-catenin antisense oligonucleotide was administered to rats after 2/3 PHx, total β-catenin decreased significantly at 24 hours.[41] Also of note was the decrease in liver weight/body weight ratio as a function of decreased proliferation in these animals. Further, the

β-catenin knockout mice mentioned previously showed a sick and lethargic phenotype after PHx as opposed to their wild-type counterparts. Additionally, these mice displayed suboptimal regeneration, with delayed regenerative onset and a biphasic trend in proliferation that peaked at day 3 and increased slightly again at day 14.[33] These results were confirmed by another laboratory that demonstrated a lack of cyclin D1 induction and a resultant delay in DNA synthesis in liver-specific β-catenin knockout mice.[42] Interestingly, subjecting TOPGAL-reporter mice to partial hepatectomy revealed that the delay in proliferation occurred despite a lack of observed β-catenin activation; thus, the usefulness of this mouse model to the study of liver processes such as regeneration remains unclear.

The function of β-catenin in liver growth and regeneration emphasizes its vital role in the health and repair of adult liver. As fetal development is also a time of increased cellular growth in the liver, many of the same gene expression patterns seen in growth and regeneration are also present in prenatal liver organogenesis.[43]

Embryonic Liver Development

The induction of embryonic liver is a complex process that requires a series of tightly regulated localized signals from multiple cell types (Fig. 2). Liver in mouse begins to arise from the definitive gut endoderm at E8.5, or the 7–8 somite stage.[44-46] It is at this time that a family of transcription factors, Foxa, specifies the endoderm to express hepatic genes.[47,48] The fibroblast growth factors FGF1 and FGF2, which are expressed in the cardiac mesoderm at this time, are responsible for initiating the expression of these liver-specific genes in the endoderm.[49] FGF8, which is important for morphogenetic outgrowth of the liver, is also expressed during this stage.[49] Endothelial cells also interact with the hepatic endoderm shortly after specification to promote morphogenesis and liver bud formation.[50] The resulting bud migrates into the septum transversum mesenchyme upon bone morphogenic protein 4 (BMP4) signaling, which is also required for hepatogenesis.[51]

The second phase of embryonic liver growth is characterized by expansion and proliferation. The cells are now considered hepatoblasts, which means that they are capable of giving rise to both major lineages of the liver, hepatocytes and biliary epithelial cells.[52] HGF, expressed in the septum transversum mesenchyme which now surrounds the liver bud, is critical for this stage of liver growth.[53] One of the most critical genes at this stage is Hex, which is maintained by FGF and BMP4 signaling in the commitment phase;[54] expression of this transcription factor is essential for hepatoblast differentiation and liver bud expansion.[55-57] Hepatoblasts or hepatic progenitors are the bipotential stem cells that will be undergoing expansion while maintaining their de-differentiated state during this stage. This event is comparable to the expansion of a lineage-restricted progenitor population in stem cell biology. Other transcription factors required for liver development at this stage include Hlx[58] and Prox1.[59] Albumin and α-fetoprotein mRNA is also being produced at this time, indicating commitment to a hepatic fate.[60] Finally, the general architecture of the liver is beginning to be established, including the formation of sinusoids and the development of hepatic vasculature.[47,61]

The final stage is characterized by the differentiation of hepatoblasts to mature, fully-functional cell types. At the center of the differentiation process are the liver-enriched transcription factors such as hepatocyte nuclear factor (HNF) transcription factors and C/EBPα, which regulate cell fate decisions in the liver.[62,63] HFN-4α is essential for differentiation toward a hepatocyte phenotype, as well as formation of the parenchyma;[64] GATA6, which regulates HFN-4α, is also required for hepatocyte differentiation.[65] HNF-6, HNF-1β and the Notch signaling pathway are required for normal development of biliary epithelia and resulting bile duct structures.[66-68] Finally, the expression of transcription factors NFκB, c-Jun and XBP-1 is necessary for growth and morphogenesis,[52] which continues until birth.

This somewhat simplistic outline of embryonic liver development is not meant to be comprehensive; we have emphasized factors such as FGF, BMP, Foxa and Hex that are either downstream targets or upstream regulators of the β-catenin pathway, while recognizing that there are many other pathways that play a role in liver embryogenesis. Nor does it take into account the complexity involved in the expression of these growth and transcription factors. Liver development is not a linear process; rather, there is significant overlap between gene expression patterns that blur the lines between one stage of liver development and the next. Additionally, activation of one gene may initiate a feedback mechanism that regulates cross-talk between different cell populations. For example, the HNFs are capable of auto-regulating their own expression as well as cross-regulating the transcription of other liver-specific genes.[52] To complicate matters, a gene may be expressed in one zone and simultaneously repressed in a neighboring zone, which makes studying the timing of gene activation in the context of a specific region of the liver particularly important. Finally, the redundancy of the system in later stages of liver development means that other genes are often able to compensate for those that have been abolished. Nonetheless, the role of β-catenin in liver development, while still being elucidated, appears to be both a negative and a positive regulator at different times during development of the liver.[69,70]

The Role of Wnt/β-catenin in Embryonic Liver Development

The Wnt pathway is now recognized as one of the major regulators of embryonic development, controlling such processes as embryonic induction, polarity and cell fate specification.[1] In *C. elegans*, *mom* genes, which are homologues of Wnt, establish polarity in the embryo by inducing endodermal cells to adopt a mesodermal fate.[71] Depleting maternal β-catenin from *Xenopus* embryos results in reduced dorsal axial structures,[72] resulting in lack of all dorsal-anterior mesoderm tissue. Even more strikingly, mice with targeted deletions of β-catenin display a block in anterior-posterior axis formation early in embryogenesis and subsequently fail to initiate gastrulation.[73] Additionally, overexpression of Wnt8c in the mouse leads to duplication of axes.[74] At the level of organ development, Wnt is important in epithelial-to-mesenchymal transition, as demonstrated by the inactivation of Wnt-4, which results in the absence of kidneys in a mouse embryo.[75]

In recent years, a plethora of evidence has emerged identifying regulation of Wnt/β-catenin signaling as a requirement for embryonic liver development as well. In fact, in Xenopus, the impact of Wnt/β-catenin signaling on liver development can be seen as early as the maternal phase, which occurs before gastrulation. Maternal Wnt/β-catenin, in conjunction with endodermally-derived TGFβ, can induce anterior endomesoderm (AE), a subset of endoderm

cells fated to form the liver.[76] Thereafter, repression of this pathway becomes necessary during the competency and commitment stage of liver development.[77] In Xenopus, β-catenin expression after gastrulation is necessary for intestinal formation in the posterior endoderm, while repression in the anterior endoderm allows for expression of Hex, which is required for liver and pancreas development. Repressing β-catenin in the posterior endoderm causes organ buds expressing liver markers to form.[78]

Several other studies have found that inhibitors of the Wnt signaling pathway can influence early liver formation. Sfrp5, an antagonist of Wnt, is expressed in the ventral foregut endoderm that gives rise to the liver at mouse E8.5,[79] resembling the expression pattern of Hex. The expression of this inhibitor may function to modulate Wnt activity by delineating borders between organs in the developing gut.[80] Further, calcineurin, a member of the Wnt/calcium signaling pathway, is involved in dorsal-side signaling that leads to the formation of liver during Xenopus embryogenesis through its interference with canonical Wnt/β-catenin signaling.[81]

Interestingly, a recent study proposes just the opposite: that β-catenin expression is required during the liver specification stage.[82] A conditional mutant of *prometheus*, a homologue of Wnt2b in zebrafish, is expressed in the mesoderm directly adjacent to the developing liver; its deletion causes a severe but transient defect in liver formation. Further analysis revealed that expression of genes such as Hex and Prox1, which are essential in hepatoblast formation, is impaired in these mutants, thus implicating these transcription factors as potential downstream targets of the β-catenin pathway.[82] This study, which suggests that the Wnt pathway is a positive regulator of liver specification, is a contradiction to the previous work demonstrating the necessity for β-catenin inhibition during the competency stage. One possible explanation could be that β-catenin is actually activated in the hepatic induction phase, which overlaps with the competence and specification stage. Another explanation, although an unlikely one, could be the difference in species. However, it is more likely that this discrepancy is more of a timing issue whereby initial repression of Wnt signaling is immediately followed by the activation of the pathway for liver outgrowth. Additional studies, perhaps using conditional knockout mice in which β-catenin is deleted before E8.5, will be needed to address this contradiction and definitively determine whether Wnt/β-catenin signaling is activated or repressed during liver specification.

Our laboratory was the first to demonstrate a mechanistic role for the Wnt/β-catenin pathway in developing liver. Livers from mouse embryos cultured in the presence of a β-catenin antisense oligonucleotide showed a decrease in proliferation and a simultaneous increase in apoptosis, two processes vital to liver development.[83] This correlated well with a subsequent study that found overexpression of β-catenin in developing chicken livers leads to a three-fold increase in liver size, which is due at least in part to an expanded hepatoblast population. Conversely, blocking β-catenin expression through overexpression of pathway inhibitors resulted in decreased liver size and altered liver shape.[84] The effect on cell proliferation noted in both cases may be due to cell cycle mediators such as cyclin D1, which is a known downstream target of β-catenin. Interestingly, an earlier study using a deletion of GSKβ demonstrated a phenotype of increased liver cell death and liver degeneration that resulted in embryonic lethality. Whether this effect was due to untimely

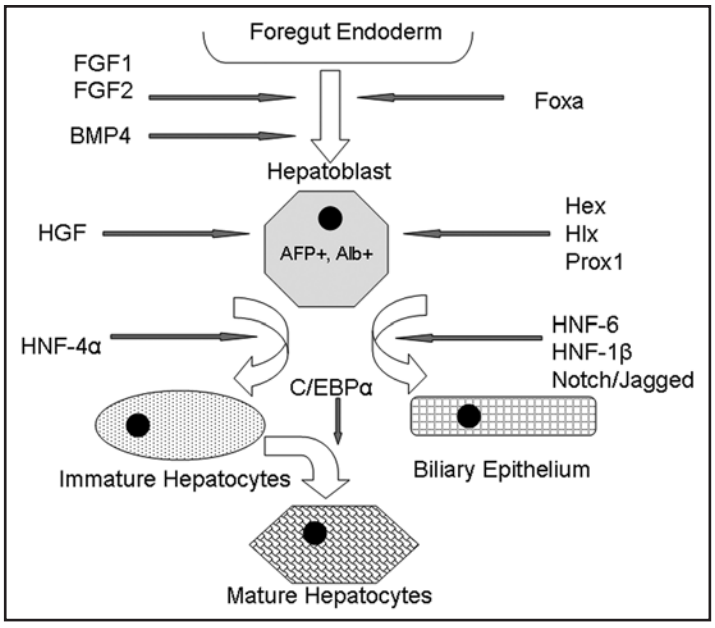

Figure 2. Model of embryonic liver development. Signals such as FGF-1, FGF-2 and BMP4 emanating from the cardiac mesoderm specify the foregut endoderm to begin expressing liver-specific genes. The Foxa transcription factors are also required for foregut endoderm specification. The resulting hepatoblasts, which are albumin and α-fetoprotein positive, proliferate and expand. Hepatoblasts express transcription factors such as Hex, Hlx and Prox1, which are essential for liver proliferation and differentiation. HNF-6, HNF-1β and the Notch/Jagged signaling pathway induce differentiation toward a biliary epithelial lineage, while HNF-4α followed by C/EBPα produces mature hepatocytes.

β-catenin stabilization or due to the fact that GSK3β is at the crossroads of several other signaling pathways critical to liver biology, such as insulin signaling, remains to be investigated further.[85] The concept of untimely β-catenin stabilization on liver growth and survival during development is also supported by a more recent study, which utilizes APC deletion during liver development. This study shows a dramatic increase in cell death and a counterintuitive decrease in cell proliferation.[86] This clearly supports a highly temporal expression, activation and role of Wnt/β-catenin signaling during the process of normal liver development and any deviation from this norm would result in abnormal growth with consequences.

Indeed, we and others have shown that β-catenin protein expression peaks at E10–12, during which time it is localized throughout the cell including the nucleus, cytoplasm and membrane. Subsequent decreases in β-catenin gene expression and increased protein degradation coincide with a dramatic decrease in total β-catenin protein expression after E16, at which time it is also localized to the membrane of maturing hepatoblasts and hepatocytes.[31,87] While the early stages coincide with ongoing hepatoblast expansion mediated via their increased proliferation and survival, the later stages represent hepatoblast maturation to hepatocytes that begin to express genes that are associates and measures of function such as transferring, cyochrome P450s, coagulation factors, haptoglobin and many others.[88]

As found in our previous studies with ex vivo embryonic liver cultures, there was also a positive correlation between β-catenin and cell proliferation, which has also been supported by additional studies in chicken, zebrafish and Xenopus.[33,78,82-84] Thus, these

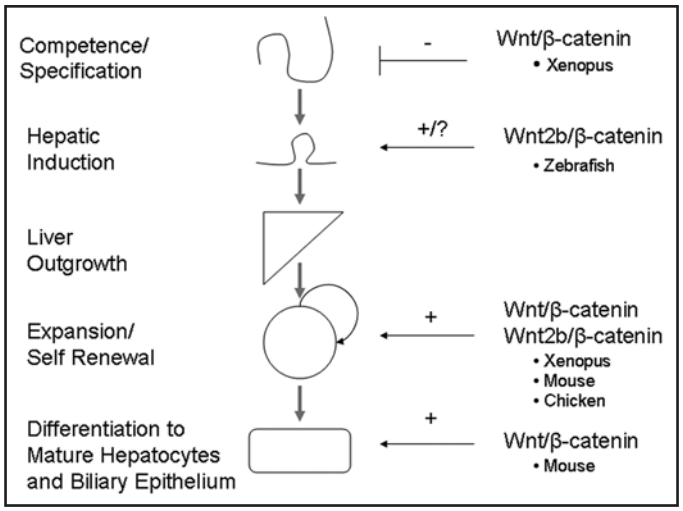

Figure 3. Role of β-catenin in embryonic liver development. β-catenin expression must be suppressed during the competence and specification stage in order for normal liver development to occur. However, expression of β-catenin is essential during the later stages of liver development, such as outgrowth, expansion and differentiation. Although there is conflicting evidence concerning the role of β-catenin during very early liver development, we hypothesize that β-catenin expression is necessary for hepatic induction immediately after its repression during the competence/specification stage. Thus, β-catenin has been found to play a role in all stages of embryonic liver development.

studies have established an important physiological role for β-catenin during early liver development in expansion of hepatoblasts or the hepatic progenitors. Interestingly, the adult counterpart of this cell is the facultative stem cell, also known as the oval cell. Oval cells are also bipotential progenitors that are activated during regeneration when hepatocyte proliferation is impaired.[89] These postnatal facultative hepatic progenitor cells have also been reported to be present in normal fetal livers.[90] Consequently, it is interesting to note that Wnt signaling is important in the induction of an oval cell response. Mice fed a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet, which enriches for oval cells, showed nuclear translocalization of β-catenin, which corresponded with an increase in proliferation, as expected.[91] Similarly, we found an increase in active β-catenin in mice fed 2-acetylaminofluorine followed by partial hepatectomy, while β-catenin conditional knockout mice fed a DDC diet had a noticeable decrease in the number of oval cells.[92] Since both normal embryonic liver development and oval cell activation after liver injury have this pathway in common, it appears that β-catenin is a shared link among many distinct physiological processes in the liver.

β-catenin also plays an important role in the differentiation of hepatoblasts into the two major liver lineage cell types: biliary epithelial cells and hepatocytes. The addition of Wnt3a to the explanted mouse embryos induced a biliary phenotype and duct-like arrangement in the developing liver, while lack of Wnt3a causes loss of architecture, proliferation and increased apoptosis.[93] Interestingly, the β-catenin activation shown recently in the APC mutant mouse also imparts a pro-biliary differentiation to the hepatoblasts, clearly verifying our previous studies.[86]

We also observed a lack of mature hepatocytes in the absence of β-catenin in our ex vivo embryonic liver cultures. This phenotype was confirmed by the concomitant presence of stem cell markers and mature hepatocyte markers in the organ culture.[83] Using another in vitro model that recapitulates hepatocyte differentiation, we found an increase in total β-catenin proteiβn as early as 24 hours after the induction of differentiation.[94] This increase was a result of decreased protein degradation and resulted in membranous localization of β-catenin rather than the nuclear localization that is the hallmark of proliferating undifferentiated cells. Finally, deletion of β-catenin from hepatoblasts in vivo using β-catenin transgenic mice under the Foxa3 promoter resulted in the decrease of liver-specific transcription factors C/EBPα and HNF4α and an overall hepatic deficiency.[95]

These studies imply that the presence of β-catenin, as well as its location inside the cell, might be a critical event dictating differentiation. This is also supported by that fact that the Wnt/β-catenin pathway has recently been labeled as the zonation keeper of the liver.[96] It regulates the expression of various genes that encode for proteins involved in ammonia metabolism, xenobiotic metabolism and others, all functions of differentiated hepatocytes.[34,35,37] Whether some of these genes are direct transcriptional targets of β-catenin or are mediated by β-catenin's role in regulating some of the key liver-specific transcription factors remains to be elucidated.

Although the picture is far from complete, the data thus far suggests that β-catenin levels vary during prenatal hepatic development, and that the temporal expression of β-catenin regulates liver formation (Fig. 3). A major undetermined aspect of Wnt/β-catenin signaling during liver development remains the obscurity of upstream effectors such as Wnt/Fz genes and related proteins that are dictating the temporal expression and activation of β-catenin. However, spatiotemporal expression of β-catenin is not unique to the liver; the Wnt/β-catenin pathway also shows distinct stage specific effects during cardiac,[97,98] gut,[99] and lens development.[100] For example, activating β-catenin expression during embryogenesis enhances cardiomyocyte differentiation, while activation of this pathway later in development causes inhibition of differentiation.[101] In the gut, Wnt expression is present in developing intestines after the appearance of villi, disappears during villi morphogenesis, and then reappears in differentiated postmitotic villus epithelium.[102] Thus, data from organogenesis studies demonstrates that turning β-catenin expression on and off at various stages of development can cause opposing results depending on the timing of induction.

The interaction of β-catenin with its other binding partners—Met and E-cadherin—can also impact liver development. We found increased association with Met during Matrigel-induced differentiation, which suggests that this interaction is crucial for hepatocyte maturation.[94] The interaction of β-catenin with E-cadherin, which is important in regulating cell-cell adhesion, increases at E16 through E18.[31] During this stage of liver development, β-catenin is principally located at the cell membrane. In fact, the ability to form intercellular adhesions, which is characterized by the Met/β-catenin and E-cadherin/β-catenin complexes, may be a marker of hepatic maturity.[17]

Studies into the mechanism of Wnt/β-catenin activation in liver development have implicated FGF proteins as upstream mediators of this pathway. Expression of FGF-10 in the mouse liver correlates with peak β-catenin activation; moreover, release of FGF-10 from stellate cells stimulates β-catenin expression in hepatoblasts.[103] Our laboratory has found that FGF-2, FGF-4 and FGF-8 promote

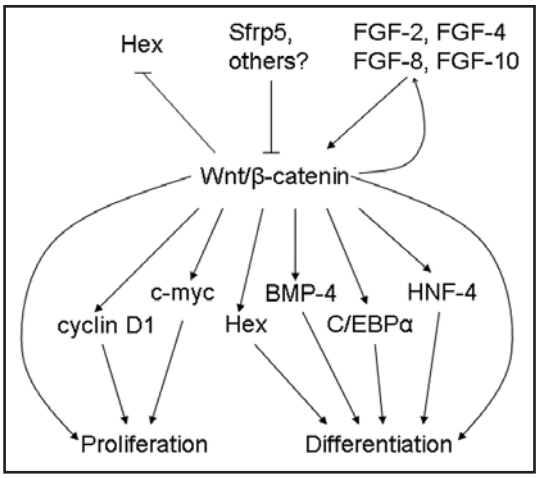

Figure 4. Upstream regulators and downstream targets of β-catenin during embryonic liver development. β-catenin expression in early foregut development inhibits Hex expression; later in hepatogenesis, β-catenin is thought to regulate proliferation in part through Hex. Srfp5 inhibits β-catenin activity, while FGFs stimulate the β-catenin pathway. In turn, β-catenin activates FGF-8 expression, suggesting a feed-forward mechanism. Downstream targets of β-catenin, such as cyclin D1 and c-myc, induce proliferation, while others, such as Hex, BMP4, C/EBPα and HNF-4, promote differentiation.

an increase in the number of hepatic progenitor cells in ex vivo embryonic livers. FGF-8 in particular seems to be crucial to the enrichment of progenitor cells as it promotes hepatocyte differentiation in addition to proliferation.[104] Addition of these growth factors to explanted livers also induced β-catenin expression, suggesting that FGF activates the Wnt pathway. Since FGF-8 is also a downstream target of the Wnt pathway,[105,106] these relationships are suggestive of a positive feedback mechanism in which β-catenin plays a central regulatory role (Fig. 4).

Conclusions

In liver regeneration, the main function of the β-catenin pathway is to regulate cell proliferation. Data thus far suggests that β-catenin plays a similar role in liver development. As liver development is physiologically similar to liver regeneration, this conclusion would seem to be in agreement with previous studies and conclusively positions β-catenin as an integral part of liver biology.

A growing body of evidence points toward a crucial role of Wnt/β-catenin in liver development. Further studies will be necessary to identify upstream regulators of Wnt, as well as downstream targets of β-catenin, in order to better appreciate the impact of this pathway on developing liver. Tools such as conditional knockout mice, gene array analysis and embryonic liver cultures will continue to enhance our understanding of this important pathway.

Acknowledgements

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