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Wnt/β-Catenin Signaling in Liver Development, Homeostasis, and Pathobiology

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Abstract

The liver is an organ that performs a multitude of functions, and its health is pertinent and indispensable to survival. Thus, the cellular and molecular machinery driving hepatic functions is of utmost relevance. The Wnt signaling pathway is one such signaling cascade that enables hepatic homeostasis and contributes to unique hepatic attributes such as metabolic zonation and regeneration. The Wnt/β-catenin pathway plays a role in almost every facet of liver biology. Furthermore, its aberrant activation is also a hallmark of various hepatic pathologies. In addition to its signaling function, β-catenin also plays a role at adherens junctions. Wnt/β-catenin signaling also influences the function of many different cell types. Due to this myriad of functions, Wnt/β-catenin signaling is complex, context-dependent, and highly regulated. In this review, we discuss the Wnt/β-catenin signaling pathway, its role in cell-cell adhesion and liver function, and the cell type–specific roles of Wnt/β-catenin signaling as it relates to liver physiology and pathobiology.

Keywords

hepatocyte; cholangiocyte; liver stem cell; regeneration; zonation; hepatocellular cancer; hepatoblastoma; liver tumors

SIGNALING

The Wnt/β-Catenin Signaling Pathway

When canonical Wnt/β-catenin signaling is inactive, the levels of the major transducer of Wnt signaling, β-catenin, are kept low via its degradation by the destruction complex (Figure 1*a*). This complex consists of the proteins Axin, adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK3β), and casein kinase 1α (CK1α) (1, 2). The scaffold

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protein Axin brings together the components of the destruction complex, mediating the phosphorylation of β-catenin first by CK1α at serine 45 and, subsequently, the phosphorylation of serines 33 and 37 and threonine 41 by GSK3β (3, 4). Phosphorylated β-catenin is recognized by β-transducin repeat-containing protein (βTRCP), a component of the E3 ubiquitin ligase complex, which triggers the ubiquitination and subsequent proteasomal degradation of β-catenin (5, 6).

Activation of the Wnt/β-catenin signaling pathway is mediated through Wnt ligands, a family of secreted glycoproteins (Figure 1*b*). To be bioactive, Wnt ligands must be glycosylated and palmitoylated by the enzyme porcupine (7). This modification occurs in the endoplasmic reticulum, and palmitoylation, the hydrophobic lipid modification, renders Wnts relatively insoluble (8). Secretion of hydrophobic Wnts from the cell requires the cargo receptor Wntless, a multipass transmembrane protein that mediates protein trafficking between the Golgi apparatus and cell membrane (9, 10). Once secreted, the Wnt ligand binds to a Frizzled receptor and the coreceptor low-density lipoprotein receptor-related protein (LRP) 5 or 6 to mediate activation of the Wnt/β-catenin signaling pathway (11–14). Wnt binding to Frizzled and LRP5/6 triggers recruitment of the scaffolding protein Dishevelled (Dvl), phosphorylation of LRP5/6, and phosphorylated LRP5/6-mediated recruitment of Axin to the plasma membrane (15, 16). Interestingly, the family of R-spondin secreted proteins enhances Wnt signaling through binding to the leucine-rich repeat-containing G protein–coupled receptor-4 (LGR4) and LGR5, which, in turn, increase Wnt-dependent phosphorylation of LRP6 (17, 18). R-spondin ligands also enhance Wnt signaling through the clearance of transmembrane E3 ubiquitin ligases zinc and ring finger 3 (ZNRF3) and its homolog ring finger 43 (RNF43), which ubiquitinate Frizzled and LRP6 and target them for proteasomal degradation (19, 20) (Figure 1). The recruitment of Axin to the plasma membrane leads to disruption of the destruction complex, promoting stabilization and cytoplasmic accumulation of β-catenin. Nonphosphorylated β-catenin is translocated to the nucleus where it forms a complex with T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to mediate expression of target genes (21).

Noncanonical Wnt Signaling

There are 19 Wnts and 10 Frizzled receptors in the mammalian genome (22), and not all of them utilize the same downstream signaling components. Certain Wnt ligands can signal independently of β-catenin, and this form of signaling is referred to as noncanonical Wnt signaling. Two classic noncanonical Wnt signaling pathways have been described: the Wnt/calcium pathway and the planar cell polarity (PCP) pathway (Figure 2).

In the Wnt/calcium pathway, noncanonical Wnt ligands, such as Wnt5a, bind to Frizzled receptors (Frizzled-2 or Frizzled-7) or with receptor tyrosine kinase-like orphan receptor 2 (Ror2) (23) (Figure 2). After Wnt binding, a complex forms among Frizzled, Dishevelled, and G proteins, which promotes the activation of phospholipase C (PLC), which cleaves phosphatidylinositol 4,5 biphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). In turn, DAG activates protein kinase C (PKC), and IP3 promotes increased intracellular calcium levels. This increase in calcium activates calcium/

calmodulin-dependent kinase II (CaMKII) and calcineurin (CaN), which regulate cell migration and proliferation (24, 25).

In the PCP pathway, Wnt ligands bind to the Ror2/Frizzled/Dishevelled complex and trigger the activation of Rho-family small GTPases, including RhoA and Rac (Figure 2). These subsequently activate Rho-associated protein kinase (ROCK) and c-Jun N-terminal kinase (JNK), which regulate cell polarity and migration (24–26). To add further complexity to Wnt signaling pathways, noncanonical Wnt5a can inhibit canonical Wnt/β-catenin signaling by promoting the degradation of β-catenin (27).

More recently, other β-catenin-independent Wnt signaling pathways have been described (Figure 3). One of these is the Wnt-dependent stabilization of proteins, known as the Wnt/STOP pathway (28). The central mediator of this pathway is GSK3β, which was found to phosphorylate many additional proteins besides β-catenin and target them for proteasomal degradation (29–32). Wnt binding to its coreceptors can trigger the sequestration of GSK3β in multivesicular bodies, allowing the cytoplasmic accumulation of GSK3β-target proteins (33–35) (Figure 3). It was recently described that Wnt/STOP signaling during mitosis slows protein degradation, stabilizes cell cycle effectors, such as c-MYC, and promotes an increase in cell size as cells prepare to divide (28). Wnt/STOP signaling is thought to play a role in many cellular processes, including cell division, regulation of the cytoskeleton, and DNA remodeling (36).

Another Wnt signaling pathway that is independent of β-catenin is Wnt-dependent regulation of mechanistic target of rapamycin (mTOR) signaling (Wnt/TOR signaling) (Figure 3). In this pathway, GSK3β phosphorylates and activates tuberous sclerosis complex 2 (TSC2), which, in turn, inhibits the function of mTOR complex 1 (mTORC1). The presence of Wnt ligands prevents GSK3β-mediated phosphorylation of TSC2, which leads to the activation of the mTORC1 signaling pathway and stimulation of protein translation (37). These studies collectively demonstrate that Wnt ligands are pleiotropic signaling molecules. The rest of this review focuses on canonical Wnt/β-catenin signaling because it is the most extensively studied Wnt signaling pathway in the context of the liver.

β-Catenin at Adherens Junctions

In addition to enacting Wnt signaling, β-catenin plays a role in cell-cell adhesion as a component of adherens junctions. Adherens junctions are subapical junctions that promote homotypic cell-cell adhesion in epithelial tissues (38). A class of transmembrane proteins called cadherins performs the extracellular interactions during cell-cell adhesion, and E-cadherin is the prototypical cadherin of adherens junctions. The extracellular domain of E-cadherin forms calcium-dependent complexes with E-cadherin molecules of neighboring cells to mediate homotypic cell-cell adhesion. β-catenin functions in adherens junctions by linking E-cadherin to the actin cytoskeleton through binding to α-catenin, which, in turn, binds directly to actin and to actin-binding proteins, such as vinculin (39). In addition, β-catenin facilitates the assembly of adherens junctions. β-catenin was shown to bind to newly synthesized E-cadherin (40) and promote delivery of E-cadherin from the endoplasmic reticulum to the basal-lateral plasma membrane (41). Later, it was shown that binding of β-catenin to E-cadherin blocks a peptide sequence, which, if exposed, would target E-cadherin

for proteasomal degradation (42). However, β-catenin is not permanently incorporated into adherens junctions. Tyrosine phosphorylation of β-catenin at residues Y142, Y654, and Y670 by the activity of hepatocyte growth factor (HGF)/c-met, Y489 by Abl, and Y654 by the epidermal growth factor receptor (EGFR) and Src may induce dissociation of β-catenin from adherens junctions and may activate β-catenin signaling (43–45). β-catenin may also play a role in the development of tight junctions, which serve to prevent bile from the bile canaliculi from mixing with blood in hepatic sinusoids (46). Catenins may participate in the trafficking of tight junctional protein zonula occludens-1 (ZO-1) from the cytosol to the plasma membrane early in tight junction development (47). Additionally, tight junctional protein claudin-2 is a transcriptional target of β-catenin (48), and depletion of claudin-2 in a polarized hepatic cell line resulted in defects in bile canalicular formation (49).

CELL TYPE–SPECIFIC ROLES OF WNT/β-CATENIN SIGNALING IN LIVER PHYSIOLOGY AND DISEASE

Wnt/β-Catenin Signaling in Hepatocytes

Hepatocytes constitute the major cell type in the liver and are responsible for performing the main functions of the liver, including synthesis, detoxification, and metabolism. The Wnt/β-catenin signaling is active in a subset of these cells constitutively, while β-catenin, as a component of the adherens junctions, is ubiquitously present in all hepatocytes. Here, we discuss the importance of this signaling cascade in hepatocytes in various aspects of hepatic pathobiology.

Wnt/β-catenin signaling in liver development—Wnt/β-catenin signaling is necessary for organismal development, as evidenced by the embryonic lethality owing to a defect in gastrulation in mice lacking β-catenin (50). During gastrulation and early somitogenesis, the endoderm is patterned into foregut, midgut, and hindgut along the anterior-posterior axis. Liver is eventually derived from the foregut endoderm. Wnts are initially essential in posterior endoderm development (51, 52). Here, FGF4 and Wnts from the surrounding mesoderm promote hindgut fate at the expense of foregut, whereas in the anterior endoderm, the suppression of these signaling molecules promotes the foregut fate (19, 51–53). Wnt signaling is repressed in the anterior endoderm by Sfrp5, a secreted Wnt inhibitor, to maintain foregut fate, and to allow for subsequent hepatic development (20). Whereas Wnt suppression is pertinent for foregut development, its subsequent activation is equally important for hepatic specification, which occurs around embryonic day 8.5 (E8.5) in mice due to the activity of transcription factors hepatocyte nuclear factor (HNF)-1β, forkhead box (Fox) A1, FoxA2, and GATA binding protein 4 (GATA4) (54–57). Low levels of fibroblast growth factor (FGF) from the adjacent cardiogenic mesoderm promote hepatic gene induction in the anterior endoderm and lead to a formation of a liver bud composed of hepatoblasts, cells that are capable of giving rise to the two main epithelial cell types of the liver, hepatocytes and biliary epithelial cells (BECs) (58). Wnt2b has been shown to induce hepatic specification at this stage because zebrafish embryos lacking Wnt2bb (homolog of Wnt2b) display a transient defect in liver specification, called the prometheus phenotype (59). Postspecification, hepatoblasts from the liver bud migrate into the septum transversum due to bone morphogenetic protein (BMP) signaling (60). HGF in the septum transversum

binds to the c-met receptor and promotes the proliferation of hepatoblasts (61), which is dependent on the homeobox factor (*Hex*) gene (62–64). β-catenin was also shown to be important for hepatoblast proliferation, survival, and maturation (65). Thus, highly spatiotemporal Wnt/β-catenin modulation is critical for hepatic organogenesis.

In later stages of liver development, hepatoblasts differentiate into either hepatocytes or BECs (also known as cholangiocytes). The exact mechanisms underlying this process are incompletely understood. However, it is known that the transcription factor HNF4α is essential for hepatoblast differentiation to hepatocytes (66), whereas CCAAT-enhancer-binding protein-α (C/EBPα) is important for hepatocyte maturation (67). Maturing hepatocytes begin to organize into chord-like structures in part via stimulation by HGF, although hepatocyte maturation is not complete by birth (54, 68).

The role of Wnt/β-catenin signaling in hepatoblast fate determination appears to be complex. Deletion of APC in hepatoblasts, leading to stabilized β-catenin, impaired hepatocyte differentiation and promoted the biliary cell fate (69). However, whereas deletion of β-catenin from hepatoblasts using a Foxa3 promoter impaired the development of primitive bile ducts, it also led to a decrease in HNF4α and C/EBPα, causing a defect in hepatocyte maturation (65). β-catenin's role in cell-cell adhesion may also be important for hepatocyte maturity (70), as the association of β-catenin with E-cadherin increases between E16 and E18 in the murine liver (71). β-catenin can also interact with c-met, and upon HGF binding to c-met, β-catenin is phosphorylated and translocates to the nucleus (44). The β-catenin/c-met pathway promotes hepatocyte proliferation and may promote hepatocyte maturation during liver development (72). Wnt/β-catenin signaling is also important for postnatal liver development, because mice with conditional loss of β-catenin in hepatocytes display a significant decrease in the liver weight/body weight ratio (73). Similarly, mice with hepatocyte-specific deletion of LRP5 and LRP6 (74), as well as mice with hepatocyte-specific deletion of LGR4 and LGR5 (75), exhibit significantly reduced liver weight. Conversely, mice with liver-specific overexpression of wild-type β-catenin have a 15% increase in liver size due to increased hepatocyte proliferation (76).

Wnt/β-catenin signaling in liver homeostasis—Because hepatocytes are the main parenchymal cell type of the liver, consisting of approximately 80% of liver mass (77), a great deal is known about the role of Wnt/β-catenin signaling in hepatocytes during both homeostasis and liver regeneration. In a baseline liver, hepatocytes display molecular heterogeneity depending on their location within the hepatic lobule (74). A liver lobule is divided into three zones: Hepatocytes near a portal triad (which consists of the portal vein, bile ducts, and hepatic artery) are labeled zone 1; hepatocytes surrounding central veins constitute zone 3; and hepatocytes in between constitute zone 2 (78) (Figure 4). The process of hepatic zonation begins in the first few weeks after birth (54). This process serves to compartmentalize opposing metabolic processes, as periportal hepatocytes perform gluconeogenesis, cholesterol biosynthesis, and urea metabolism, whereas pericentral hepatocytes perform glycolysis, bile acid biosynthesis, and glutamine synthesis (79). Wnt/β-catenin signaling has proven to be a master regulator of liver zonation. Pericentral hepatocytes show active Wnt/β-catenin signaling as demonstrated by pericentral-specific expression of Wnt/β-catenin targets Axin2 (80), glutamine synthetase (GS), and cytochrome

P450 enzymes CYP2E1 and CYP1A2 (73, 81) (Figure 4). Importantly, mice with liver-specific deletion of both LRP5 and LRP6 (74), as well as mice with hepatocyte-specific deletion of LGR4 and LGR5 (75), lack liver zonation and fail to express the pericentral metabolic genes. Mice with inducible loss of β-catenin in hepatocytes display a periportal phenotype throughout the whole liver (82). In contrast, overactivation of β-catenin specifically in hepatocytes due to conditional APC deletion led to a pericentral phenotype throughout the entire hepatic lobule (83). These results are explained by the finding that HNF4α and β-catenin compete for binding to TCF; HNF4α/TCF dictates periportal gene expression whereas β-catenin/TCF dictates pericentral gene expression (82) (Figure 4). This hypothesis is supported by the finding that HNF4α-deficient livers exhibit a pericentral phenotype (84). Hepatocyte differentiation is also key for metabolic zonation. In mice with liver-specific Yes-associated protein (YAP) deletion, GS-positive cells expanded in the pericentral domain. Alternatively, in mice with liver-specific deletion of macrophage stimulating 1 (Mst1)/Mst2, the kinases responsible for phosphorylation and inactivation of YAP, metabolic zonation was disrupted, with loss of expression of pericentral genes, such as GS. YAP overexpression led to dedifferentiation of hepatocytes, with downregulation of HNF4α target genes and promotion of a stem cell–like phenotype (85). Yap was found to be normally expressed in the periportal domain, which suggests that it functions in opposition to Wnt/β-catenin signaling. These results collectively demonstrate the important role of Wnt/β-catenin signaling in baseline hepatic function.

Wnt/β-catenin signaling in liver regeneration and metabolic diseases—A notable feature of the liver is that normal cell turnover and liver regeneration following most models of acute liver injury are mediated by the proliferation of existing differentiated hepatocytes (86). A well-studied model of liver regeneration is the two-thirds partial hepatectomy (PHx) model, in which two-thirds of a rat or mouse liver are removed and within days the remaining lobes enlarge to replace lost liver mass via mostly cellular hyperplasia and some through cellular hypertrophy (87). Wnt/β-catenin signaling is an important driver of liver regeneration in this model; within minutes of PHx in the rat there is a transient 2.5-fold increase in β-catenin protein, which rapidly translocates to the nucleus (88). This increase in nuclear β-catenin helps to promote hepatocyte proliferation through the expression of target genes, such as cell-cycle regulator cyclin D1, which increases expression as early as 6 h post-PHx (89) (Figure 5). Interestingly, mice with hepatocyte-specific loss of β-catenin display a delay in regeneration following PHx, as there was a twofold reduction in proliferating hepatocytes at the 40-h post-PHx time point, which is the peak of hepatocyte proliferation in the wild-type mice. However, there was a subsequent increase in hepatocyte proliferation 3 days post-PHx, which indicated an activation of a compensatory signaling pathway (73). Mice lacking both Wnt coreceptors LRP5 and LRP6 in hepatocytes exhibit a similar delay in liver regeneration after PHx (74), as do mice lacking both LGR4 and LGR5 in hepatocytes (75). Interestingly, noncanonical Wnt signaling was shown to be involved in the conclusion of liver regeneration post-PHx. Wnt5a was found to inhibit canonical Wnt/β-catenin signaling in cultured primary hepatocytes, and mice with liver-specific deletion of Wntless displayed continued hepatocyte proliferation for up to 4 days longer than control littermates, owing to the reduced expression of inhibitory Wnt5a

24–48 h post-PHx (90) (Figure 5). This suggests an autocrine mechanism of proliferation termination following the acquisition of required hepatocyte mass during regeneration.

A common cause of acute liver injury in patients is overdose of acetaminophen (APAP) leading to hepatotoxicity (91). In the liver, APAP is metabolized by the enzymes CYP2E1 and CYP1A2 into a reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI), which covalently binds to cellular macromolecules and induces hepatic necrosis (92). Both *CYP2E1* and *CYP1A2* are β-catenin target genes, so mice with hepatocyte-specific β-catenin loss are resistant to APAP-induced hepatotoxicity (81). However, β-catenin may also promote liver regeneration following APAP overdose, as liver-specific β-catenin knockout mice given APAP following induction of CYP1A2 and CYP2E1 exhibited significant defects in hepatocyte proliferation following APAP-induced hepatic necrosis (93).

A role for Wnt/β-catenin signaling has also been implicated in ischemia/reperfusion injury. Under conditions of hypoxia, hypoxia inducible factor-1α (HIF1α) directly competes with TCF4 for binding to β-catenin, which leads to an enhancement of HIF1α-mediated transcription and the promotion of cell survival (94). Mice with β-catenin-deficient hepatocytes displayed reduced HIF1α signaling and were more susceptible to ischemia/reperfusion injury, whereas mice with hepatocyte-specific Wnt1-overexpression had enhanced HIF1α signaling and were protected (95).

Deficient Wnt/β-catenin signaling may also exacerbate the development of hepatic steatosis. Loss-of-function point mutations in LRP6 have been identified in humans with early onset cardiovascular disease, hyperlipidemia, and metabolic syndrome traits (96). Mice with mutant LRP6 develop fatty liver due to increased AKT/mTOR signaling causing elevated hepatocyte lipogenesis, which can be normalized through exogenous Wnt3a treatment (97). Additionally, β-catenin has been found to regulate hepatic mitochondrial homeostasis, as mice with β-catenin-deficient hepatocytes subjected to acute ethanol intoxication displayed reduced mitochondrial function in addition to impaired Sirtuin 1 (Sirt1)/peroxisome proliferator-activated receptor α (PPARα) signaling, leading to increased steatosis and oxidative damage (98). The role of Wnt/β-catenin signaling in hepatic metabolism was further expanded by the discovery of the interaction of β-catenin and forkhead box protein O (FOXO) transcription factors. Under conditions of oxidative stress, β-catenin binds directly to FOXO and enhances transcription of FOXO target genes (99). It was also found that β-catenin modulated hepatic insulin signaling, and the association of β-catenin and FOXO1 was promoted in mice under starved conditions. Interestingly, β-catenin and FOXO1 promoted the expression of rate-limiting enzymes in hepatic gluconeogenesis, and the liver-specific deletion of β-catenin in mice fed a high-fat diet displayed increased glucose tolerance due to decreased gluconeogenesis (100). Collectively, these results demonstrate the importance of Wnt/β-catenin signaling in hepatic metabolism and could implicate a role of this pathway in the pathogenesis of conditions such as nonalcoholic fatty liver disease.

Wnt/β-catenin may also play a role in bile acid secretion and homeostasis. Hepatocytes are responsible for the conversion of cholesterol into bile acids, which are secreted into bile canaliculi for eventual transport to the lumen of the small intestine to aid in the digestion of dietary lipids and cholesterol (101). Two of the key enzymes in bile acid biosynthesis,

CYP7A1 and CYP27, are expressed in pericentral hepatocytes, which suggests that they are regulated by Wnt/β-catenin signaling (102). Mice with liver-specific deletion of β-catenin fed a methionine-choline-deficient diet to induce liver injury displayed significant steatohepatitis, accumulation of hepatic cholesterol and bile acids, and elevated serum bilirubin, suggesting a defect in bile acid export (103). Furthermore, mice with liver-specific deletion of β-catenin displayed dilated and tortuous bile canaliculi and reduced bile flow rates, and feeding these mice a diet supplemented with cholic acid to induce bile acid–mediated liver toxicity led to the development of intrahepatic cholestasis and fibrosis (48). These results suggest that aberrant Wnt/β-catenin signaling may play a role in the development of cholestatic liver disease.

Wnt/β-catenin signaling in liver tumors—Despite decades of research, the incidence of liver cancer continues to rise and it remains one of the most fatal cancers (104). Liver cancer is the sixth most common cancer and third leading cause of cancer death worldwide (105). Approximately 70–90% of these cases are hepatocellular carcinoma (HCC) (105), of which β-catenin activation is observed in 20–35% of cases. The most common mutations occur in the gene encoding β-catenin, *CTNNB1*, in exon 3 around serine/threonine sites, which prevent the proteasomal degradation of β-catenin and lead to its stabilization (106). Consistent with the proposed role for Wnt/β-catenin signaling in biliary homeostasis, HCC with activation of β-catenin displays intratumoral cholestasis (107). These tumors are also strongly positive for GS, a surrogate β-catenin target (108–110). Interestingly, β-catenin activation alone is insufficient to promote HCC in mouse models, as mice with liver-specific overexpression of wild-type, serine 45-mutant, or exon-3-deleted β-catenin fail to develop spontaneous liver cancer (76, 111, 112). However, mice with liver-targeted acute APC loss developed HCC with strong activation of β-catenin signaling (113). Indeed, mice with the activation of β-catenin signaling are more susceptible to diethylnitrosamine (DEN)-induced hepatocarcinogenesis (111, 114). It has also been shown that β-catenin can cooperate with other oncogenes to drive the development of HCC. Mice with simultaneous liver-targeted mutations of β-catenin and H-Ras developed HCC, whereas mice with a single mutation of β-catenin or H-Ras alone failed to develop tumors (115). In HCC patients, exome sequencing has also revealed cooperating mutations of *CTNNB1* with *ARID2*, *NFE2L2*, *TERT*, *APOB*, and *MLL2* (Figure 6*a*) (116). Likewise, certain mutations were also always mutually exclusive from *CTNNB1* and demonstrated a lack of cooperation in the development of HCC. These included *TP53* and AXIN1 (Figure 6*a*) (116). The sleeping beauty transposon/transposase system combined with hydrodynamic tail vein injection (SB-HTVI) in mice has been utilized to demonstrate true cooperation and functionality of β-catenin with some of the other proto-oncogenes (117). Because Met overexpression/activation and mutations in β-catenin were also found in approximately 9–12.5% of all HCC patients, these two proto-oncogenes were coexpressed using SB-HTVI. These mice developed HCC with gene expression profiles that displayed high correlation with the gene profiles of a subset of human HCC patients with both *CTNNB1* mutations and Met activation signatures (118). To address if Ras activation downstream of Met could be contributing to Met-β-catenin HCC, G12D-*KRAS* and mutant-β-catenin were expressed using SB-HTVI, which also yielded HCC with approximately 90% molecular similarity to Met-β-catenin HCC (119). In fact, treatment of these mice with lipid nanoparticles

containing small interfering RNA (siRNA) targeting *CTNNB1* yielded a significant decrease in tumor burden (119). Thus, targeting β-catenin in HCC may prove to be part of a viable treatment strategy, as a course of antisense-mediated β-catenin suppression treatment led to a complete therapeutic response of β-catenin-activated HCC in mice (120). Therapeutic targeting of β-catenin in select HCCs may be effective because it regulates the expression of an array of downstream targets with diverse functions in tumor proliferation, survival, metabolism, immune tolerance, and angiogenesis, as reviewed elsewhere (Figure 6*b*) (106).

Another type of liver cancer is hepatoblastoma (HB), which is the most common malignant liver tumor in children. DNA aberrations affecting exon 3 in β-catenin in the form of deletions or missense mutations are known to occur in the majority of sporadic HB (121). Subsequent studies have detected histological evidence of β-catenin activation in up to 87% of tumor samples (122). In the same study, up to 83% of tumor samples were positive for Y654-phosphorylated β-catenin, a hallmark of HGF/c-met activation (122). Interestingly, it was recently described that around 80% of HB tumor samples displayed simultaneous nuclear accumulation of β-catenin and YAP. Co-expression of constitutively active β-catenin and constitutively active YAP via SB-HTVI in hepatocytes led to the development of HB in mice, suggesting a role of β-catenin-YAP interaction in the pathogenesis of HB (123) (Figure 6*a*). How these two signaling pathways cooperate in HB development remains under investigation and is also discussed elsewhere (124). An intriguing observation was that, unlike in HB, less than 4% of HCC samples exhibited concomitant activation of β-catenin and YAP, although YAP and β-catenin activation was evident exclusively in a notable subset of HCC cases (123) (Figure 6*a*). Deletion of Mst1/Mst2, the kinases responsible for phosphorylation and inactivation of YAP, led to the development of HCC with a lack of nuclear β-catenin staining. Interestingly, mice with liver-specific deletion of both Mst1/Mst2 and YAP displayed characteristic β-catenin activation. Mice with YAP overexpression–mediated HCC treated with YAP siRNA led to activation of β-catenin in tumor nodules (85), which suggests that YAP activation and β-catenin activation are mutually exclusive in HCC. These results suggest distinct mechanisms of hepatocarcinogenesis involving β-catenin and YAP in HB and HCC.

Wnt/β-Catenin Signaling in Biliary Epithelial Cells

Bilary epithelial cells (BECs), or cholangiocytes, line the bile ducts. BECs are the second major type of epithelial cell of the liver, lining the intra- and extrahepatic bile ducts and constituting the biliary tree. Wnt/β-catenin signaling is also known to play an important role in various aspects of biliary physiology and pathology, which are discussed in this section.

Wnt/β-catenin signaling in bile duct development—BECs, like hepatocytes, are derived from a common progenitor during hepatic development, the hepatoblast. Hepatoblasts differentiate into either hepatocytes or BECs based on spatiotemporal signals. Hepatoblast differentiation to BECs depends chiefly on HNF6 (125), HNF1β (126), and Notch signaling (127). Additionally, it was found that activity of YAP/transcriptional coactivator with PDZ-binding motif promoted hepatoblast differentiation to BECs while suppressing hepatocyte differentiation via repression of HNF4α (128). Hepatoblast differentiation to BECs occurs near branches of the developing portal vein. The developing

biliary cells form the ductal plate, or a cell monolayer encircling the periportal mesenchyme (129). During ductal morphogenesis, the ductal plate becomes bilayered to form the primitive ductal structure (129, 130). The cell layer adjacent to the portal vein expresses mature BEC markers, whereas the cells of the layer adjacent to the parenchyma resemble hepatoblasts because they express HNF4α (129). Recent evidence has confirmed that cells of the ductal plate give rise to both BECs, which line intrahepatic bile ducts, and periportal hepatocytes (131).

The role of Wnt/β-catenin signaling in hepatoblast fate determination to BECs is not fully understood. The earliest studies using antisense-mediated suppression of β-catenin in embryonic liver cultures showed a dearth of biliary specification (116). Deletion of APC in hepatoblasts, leading to stabilized β-catenin, impaired hepatocyte differentiation and promoted the biliary cell fate (69). Deletion of β-catenin from hepatoblasts using a Foxa3 promoter impaired the development of primitive bile ducts (65). However, more recent studies using stage-specific in vivo gain- and loss-of-function approaches and more specific biliary markers demonstrated that β-catenin is dispensable for the differentiation of hepatoblasts to BEC precursors in mice (117). Furthermore, when β-catenin was depleted in BEC precursors using sex-determining region Y box 9 (Sox9)–driven Cre recombinase (Cre), BEC maturation, bile duct morphogenesis, and differentiation of periportal hepatocytes from BEC precursors appeared to be normal. However, an untimely stabilization of β-catenin in cholangiocyte precursors still perturbed duct development and cholangiocyte differentiation. Thus, this study concluded that β-catenin could be dispensable for biliary development, although when β-catenin is present during the development of bile ducts, its activity must be tightly regulated. It is relevant to point out that loss of β-catenin in Sox9-positive cells could be compensated by another mechanism, such as γ-catenin, which is upregulated upon β-catenin loss (132, 133).

Wnt/β-catenin signaling in liver progenitor cells—BECs line the bile ducts to form a network of tubular conduits that function to collect, store, and secrete bile into the duodenum (134). In addition, BECs are thought to be the cell compartment that gives rise to liver progenitor cells (LPCs or oval cells), which mediate liver regeneration under conditions of extreme liver injury. Because liver regeneration is usually contingent on the proliferation of hepatocytes, it has been proposed that when hepatocyte proliferation is impaired, bipotent LPCs proliferate and differentiate into hepatocytes to mediate liver regeneration (86, 135) (Figure 7*a*). LPCs are not detected in normal livers (136) and are thought to arise from cells in the canal of Hering (131, 135, 137), the hepatocyte-biliary interface of bile canaliculi and terminal bile ducts, which contains small hepatocytes with the expression of both BEC-marker cytokeratin 19 and albumin (137) and BECs with the expression of hepatocyte markers such as HNF4α (138). Many studies have demonstrated that LPCs are derived from BECs using the choline-deficient, ethionine-supplemented (CDE) diet, a model of liver injury that promotes expansion of LPCs (139). In mice with tamoxifen-inducible Sox9-Cre to label cells originating from the ductal plate, it was found that ductal plate cells gave rise to adult BECs, periportal hepatocytes, and oval cells in CDE diet–induced liver injury (131). Similarly, mice with labeling of ductal plate cells with yellow fluorescent protein (YFP) via osteopontin-driven Cre displayed YFP-positive LPCs after exposure to a CDE diet (140).

Labeling of BECs with YFP via HNF1β-driven Cre also yielded YFP-positive LPCs in mice given a CDE diet (141).

Several animal models have shown that LPCs can differentiate into hepatocytes to mediate liver regeneration (Figure 7*a*). When hepatocyte proliferation in the rat was blocked through administration of 2-acetylaminofluorene (AAF) followed by PHx, there was a massive expansion of oval cells from the periportal region into the parenchyma, and these cells were shown to give rise to hepatocytes (142, 143). Several studies in mice with YFP labeling of LPCs have reported that 2–30% of hepatocytes were YFP-positive after a CDE diet–induced liver injury (140, 141, 144), with no detection of YFP-positive hepatocytes from other forms of liver injury, such as PHx or chronic toxic injury from carbon tetrachloride (CCl_4) (140). However, several reports have indicated that LPCs do not contribute to liver regeneration. Studies performed using genetic fate tracing of hepatocytes or BECs in mice exposed to a CDE diet found a negligible number of hepatocytes derived from nonpreexisting hepatocytes (145–147). A potential reason for the discrepancies in these data is that the CDE diet does not block the proliferation of hepatocytes (145). A recent study achieved a robust block of hepatocyte proliferation in mice via conditional deletion of mouse double minute 2 homolog (Mdm2; an E3 ubiquitin ligase that degrades p53) specifically in hepatocytes, resulting in the accumulation of p53 and p21, hepatocyte death, and hepatocyte senescence (148). These mice displayed extensive proliferation of oval cells and repopulation of the liver with Mdm2-containing hepatocytes, providing additional evidence that LPCs can differentiate into hepatocytes to mediate liver regeneration when hepatocyte proliferation is blocked.

Wnt/β-catenin signaling has also been implicated in the differentiation of LPCs into hepatocytes. In rats treated with AAF/PHx, there was a notable increase in active β-catenin during the oval cell proliferation stage, and oval cells stained positively for nuclear β-catenin (149). A separate study demonstrated that LGR5-positive cells appeared near bile ducts after liver damage in mice, and these LGR5-positive cells could give rise to BECs and hepatocytes. Notably, LGR5 is a Wnt target gene not detected in normal livers and is the receptor for Wnt agonist R-spondin1, implying Wnt signaling–driven regeneration mediated by the LRG5-positive LPCs (150). Finally, in mice fed a CDE diet, expression of the Notch ligand Jagged-1 promoted Notch signaling and biliary differentiation in LPCs, whereas macrophage-derived Wnt3a induced canonical Wnt/β-catenin signaling in LPCs and promoted their differentiation to hepatocytes (151) (Figure 7*a*). These studies all point to a role of Wnt/β-catenin signaling in promoting LPC differentiation to hepatocytes.

Wnt/β-catenin signaling in hepatocyte–biliary epithelial cell transdifferentiation—An alternative, although not exclusive, theory against the existence of a dedicated population of LPCs proposes that BECs and hepatocytes function as facultative stem cells, or differentiated cells that may function as stem cells after injury (152). Therefore, BECs transdifferentiate to hepatocytes, and vice versa, to mediate liver regeneration. One study demonstrated that BECs can function as bipotent stem cells, as BECs isolated from human livers were expanded as epithelial organoids in culture. These biliary organoids differentiated into functional hepatocytes when transplanted into mice treated with CCl_4 and retrorsine to block hepatocyte proliferation (153). Hepatocytes have also been shown to differentiate into BECs during biliary injury. In dipeptidyl peptidase IV

(DPPIV)-negative rats transplanted with DPPIV-positive hepatocytes subjected to bile duct ligation (BDL) to induce severe biliary injury, donor DPPIV-positive hepatocytes transdifferentiated into BECs and formed ductules (154).

The appearance of biliary markers in hepatocytes has been shown to be associated with chronic liver injuries in patients (155). Similarly, in mice, biliary markers like A6 appear in hepatocytes in predominantly biliary injury models, such as mice fed a hepatotoxin 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet (156). In fate-tracing studies, hepatocytes were more convincingly shown to transdifferentiate into BECs during biliary injury and oval cell expansion during DDC diet feeding in mice (157, 158). YAP signaling activation has been shown to promote dedifferentiation of hepatocytes into a progenitor cell-like state, and these dedifferentiated hepatocytes have been shown to form ductal structures expressing markers of BECs (159).

Evidence exists for a role of Wnt/β-catenin signaling in hepatocyte–BEC transdifferentiation. Transgenic mice expressing a stable-mutant form of β-catenin in hepatocytes and fed a DDC diet for 5 months exhibited significantly lower serum alkaline phosphatase, a marker of cholestasis (160). These transgenic mice exhibited a dramatic increase in the numbers of A6-positive hepatocytes, which constituted almost the entire hepatic lobule as compared to controls, which showed only limited and localized periportal presence of A6-positive hepatocytes. A more recent study showed that this transdifferentiation corresponded with increased YAP signaling (161). This study further strengthened the role of Wnt signaling in hepatobiliary repair, as mice fed a DDC diet exhibited a significant upregulation of Wnt7a, which induced Sox9 expression and increased β-catenin reporter activity in cultured hepatocytes. Upregulation of Wnt7b and Wnt10a was also observed, and these Wnts promoted proliferation of BECs, but in a β-catenin-independent manner (Figure 7*b*). DDC-diet-fed mice with liver-specific deletion of Wntless, which prevents Wnt secretion from hepatocytes and BECs, displayed reduced BEC proliferation and abrogated hepatocyte–BEC transdifferentiation. Finally, mice with liver-specific deletion of LRP5 and LRP6 subjected to BDL, another model of chiefly biliary injury, exhibited similar levels of BEC proliferation compared to wild-type littermates but lacked transdifferentiating hepatocytes (161). All these results suggest that BEC proliferation postbiliary injury is Wnt-dependent but β-catenin-independent, and Wnt/β-catenin signaling plays a role in promoting hepatocyte–BEC transdifferentiation.

Wnt/β-Catenin Signaling in Liver Endothelial Cells

In addition to the endothelial cells that line the central veins, portal veins, and hepatic arteries, other endothelial cells line the sinusoids of the liver, termed liver sinusoidal endothelial cells (LSECs) (Figure 4). The microarchitecture of the liver optimizes its metabolic functions and allows the hepatocytes between portal triads and central veins to be constantly perfused with blood flowing through the sinusoids. LSECs lack a basement membrane and contain nondiaphragmed fenestrae (162), which act as a dynamic filter and allow only molecules smaller than the fenestrae to reach the hepatocytes (163). Additionally, LSECs are efficient endocytic cells (164), allowing them to scavenge molecules from the blood and transport them to the hepatocyte surface (165). This process allows for more

efficient delivery of metabolic compounds to the hepatocytes and also causes LSECs to function as sentinels for the hepatic acute phase response (166). LSECs express Toll-like receptors (TLRs) and can react to TLR ligands, such as bacterial-derived lipopolysaccharide (LPS) (167). As LSECs are constantly bathed in bacterial degradation products from the portal venous blood, LSECs secrete inflammatory cytokines, such as interleukin-6 (IL-6), upon changes in LPS concentration (168). Secretion of IL-6 by LSECs promotes the hepatic acute phase response, in which hepatocytes secrete complement factors to help mount an innate immune response against infection (166). Thus, LSECs promote the metabolic function and health of a normal liver.

Endothelial cells within the liver have also been implicated in liver development, maintenance of metabolic zonation, and liver regeneration. Deletion of Wntless from endothelial cells in mice was embryonic-lethal, implying the importance of the secretion of Wnt ligands from endothelial cells in organismal development (74). It was shown that Wnt signaling enhancer R-spondin3 is expressed in the endothelial cells of the central vein, and conditional deletion of R-spondin3 in mice resulted in loss of pericentral zonation markers (169). Additionally, central vein endothelial cells were found to express Wnt2 and Wnt9b, and inducible deletion of Wntless from endothelial cells resulted in loss of pericentral zonation markers, such as GS (170) (Figure 4).

Endothelial cells within the liver are also thought to participate in liver regeneration through the secretion of mitogenic factors. Deletion of endothelial cell–specific transcription factor inhibitor of DNA binding 1 in mice impaired hepatocyte proliferation 48 h post-PHx, and this decrease in hepatocyte proliferation was attributed to decreased LSEC expression of angiocrine signals Wnt2 and HGF (171) (Figure 5). These results clearly demonstrate the important role of endothelial cell–derived Wnt ligands in liver homeostasis and regeneration.

Wnt/β-Catenin Signaling in Hepatic Stellate Cells

Hepatic stellate cells (HSCs) are mesenchymal cells located in the space of Disse, the gap between hepatocytes and liver sinusoidal endothelium (172) (Figure 4). HSCs have many functions in both hepatic homeostasis and liver regeneration. HSCs are known to exist in two different states, quiescent or activated, depending on the condition of the surrounding liver. Quiescent HSCs function to store vitamin A (173) and express the markers desmin (174) and glial fibrillary acidic protein (GFAP) (175). Under conditions of liver injury, HSCs are activated and transdifferentiate into myofibroblasts expressing α-smooth muscle actin (αSMA) (176). Upon activation, HSCs are the primary extracellular matrix (ECM)-producing cells of the liver and function in wound healing to produce a scar at the site of liver injury. Additionally, HSCs produce proinflammatory cytokines and growth factors, such as HGF, to promote liver regeneration (177) (Figure 5). HSCs may also play a role in hepatic immune function, as HSCs can function as antigen-presenting cells and can modulate T-lymphocyte proliferation (178). However, the HSC is most well-known for its role in the promotion of hepatic fibrosis.

Liver fibrosis is a widespread condition characterized by chronic liver injury with concomitant accumulation of ECM proteins, such as collagen. Many forms of chronic liver injury can lead to the development of liver fibrosis, including viral hepatitis, chronic alcohol

abuse, and nonalcoholic steatohepatitis. In most forms of chronic liver injury, iterative cycles of injury damage the parenchyma of the liver, leading to inflammation, matrix deposition, angiogenesis, and progressive fibrosis (179). If left unchecked, these repeated cycles of cell death and repair can lead to the development of cirrhosis, HCC, and liver failure (179–182). This is a major public health concern. In 2013, chronic liver disease and cirrhosis were the twelfth leading cause of death in the United States (183). Prolonged activation of HSCs results in excess ECM deposition and promotes the development of liver fibrosis (177), making HSCs an attractive target for antifibrotic therapies.

Wnt/β-catenin signaling has been implicated in the process of HSC activation. Conditional deletion of β-catenin in the mesenchyme during liver development led to increased expression of αSMA in HSCs and increased collagen deposition in the developing liver (184). Additionally, it was found that Wnt/β-catenin signaling was active in freshly isolated rat HSCs. Promotion of Wnt/β-catenin signaling in cultured HSCs through a GSK3β inhibitor–impeded expression of αSMA, promoted GFAP expression, and impaired HSC proliferation, which indicates that Wnt/β-catenin signaling promotes HSC quiescence (185). However, Wnt/β-catenin signaling has also been reported to promote HSC activation. Knockdown of β-catenin expression via siRNA in a rat HSC cell line inhibited the synthesis of collagen, impaired cell proliferation, and induced apoptosis (186). Increased nuclear β-catenin was detected in culture-activated HSCs, and mice subjected to BDL to induce liver fibrosis and injected with an adenoviral vector–expressing Wnt signaling antagonist Dickkopf-1 displayed significantly reduced periportal fibrosis compared to control animals (187). A gene expression analysis of culture-activated HSCs detected upregulation of ligands Wnt4 and Wnt5a as well as coreceptors Frizzled-1 and Frizzled-2 in the absence of β-catenin activation, which suggests a role for β-catenin-independent noncanonical Wnt signaling in HSC activation (188). Consistent with these results, a more recent study found that overexpression of Wnt5a in an HSC cell line increased expression of proinflammatory cytokines and collagen while also promoting cell proliferation independent of the canonical Wnt/β-catenin signaling pathway (189). More studies will be necessary to address these discrepancies and elucidate the roles of Wnt/β-catenin signaling in HSC quiescence or activation.

Wnt/β-Catenin Signaling in Kupffer Cells

Kupffer cells are tissue-resident macrophages located within the liver. They are adherent to endothelial cells and reside within the hepatic sinusoids in close proximity to hepatocytes (190). Although Kupffer cells are liver-specific macrophages, their origin is still controversial. In general two hypotheses exist: The first hypothesis suggests that Kupffer cells do not self-renew and are derived from bone-marrow-derived monocytes that infiltrate the liver and differentiate into macrophages. The second hypothesis promotes the idea that Kupffer cells can proliferate to self-renew or are derived from an intrahepatic progenitor (191). Additionally, multiple subsets of Kupffer cells have been identified within the liver (192), although discussion of the different functions of these subsets is outside the scope of this review. In general, Kupffer cells act as sentinels, as they are the first immune cell in the liver to encounter bacteria, bacterial endotoxins, and microbial debris originating from the gastrointestinal tract transported to the liver through portal vein circulation (193). Along

with dendritic cells and LSECs, Kupffer cells constitute the reticuloendothelial system, which functions to clear antigens and pathogen-associated molecular patterns from the bloodstream and to degrade bloodborne toxins (191). Kupffer cells have long been known to play an important role in the rapid clearance of bacteria from the bloodstream (194). In a healthy liver, Kupffer cells exhibit a tolerogenic phenotype to prevent excessive immune activation in the face of gut-derived bacterial products.

In disease conditions, Kupffer cells may become activated, resulting in hepatocellular damage (190). In chemical-induced liver injury, such as administration of CCL_4, endotoxin, or APAP, Kupffer cells are a major source of inflammatory cytokines, chemokines, and free radicals (195). Activation of Kupffer cells is thought to play a major role in the development of hepatic fibrosis. Activated Kupffer cells produce transforming growth factor-β1 (TGFβ1) (196), which is thought to be the main cytokine that drives liver fibrosis in animal models, such as CCL_4-induced liver damage (197). Indeed, depletion of Kupffer cells via gadolinium chloride in rats treated with phenobarbital and CCL_4 prevented increases in TGFβ1 expression and attenuated liver fibrosis (198). Selective macrophage depletion in CCL_4-induced advanced liver fibrosis in mice reduced scarring and myofibroblast activation (199). Kupffer cell–derived TGFβ1 activated HSCs via TLR4 activation during LPS challenge in mice and promoted hepatic fibrogenesis (200). Interestingly, HSC-derived Wnt5a was shown to induce expression of TGFβ1 in cultured primary rat Kupffer cells, implying a role of Wnt signaling in Kupffer cell activation (201).

Kupffer cells are also thought to play an important role in liver regeneration. Selective depletion of macrophages during recovery from CCL_4-induced liver injury perpetuated liver fibrosis through a lack of ECM degradation (199), as Kupffer cells are a known source of matrix metalloproteinases, which can promote the resolution of fibrosis (190). Mice depleted of Kupffer cells through liposome/clodronate treatment exhibited increased sensitivity to APAP-induced liver injury, which was shown to be a result of reduced Kupffer cell–derived hepato-protective and inflammation-resolving cytokines, such as IL-6 and IL-10 (202). Kupffer cells may also actively promote liver regeneration in certain liver injury models. Mice with macrophage-specific deletion of Wntless exhibited reduced cyclin D1 expression and significantly lower hepatocyte proliferation 40 h post-PHx but not at 72 h post-PHx, implying a role of macrophage-derived Wnts in driving early liver regeneration (74). Providing further evidence for this concept is the observation that Wnt expression in macrophages is promoted by cytokines, such as TNFα, secreted from infiltrating inflammatory cells during liver regeneration (203) (Figure 5). Kupffer cells also promote liver regeneration through promoting LPC activation and differentiation into hepatocytes. Macrophage-derived Wnt3a induced canonical Wnt/β-catenin signaling in LPCs and promoted their differentiation to hepatocytes (151) (Figure 7*a*). In the rat AAF/PHx model, depletion of macrophages with clodronate prior to surgery resulted in a reduced oval cell response (204). However, in the CDE-diet model of liver injury, depletion of Kupffer cells via clodronate did not reduce oval cell proliferation in CDE-diet-fed mice but reduced oval cell invasion into the parenchyma (205). Thus, macrophage-derived Wnt ligands are important in promoting liver regeneration in multiple models of liver injury.

CONCLUSIONS AND FUTURE DIRECTIONS

As more knowledge about the role of Wnt/β-catenin signaling in liver biology is obtained, it is becoming clear that Wnt/β-catenin signaling is involved in almost every aspect of liver function, from liver development to metabolism to regeneration. This review has also illustrated the diverse roles of Wnt/β-catenin signaling in nearly every cell type in the liver. As this signaling pathway can promote both liver injury and regeneration, therapies targeting Wnt/β-catenin signaling must be carefully evaluated to consider both the disease context and the targeted cell type. For example, targeting Wnt/β-catenin signaling may be a viable therapeutic option for liver cancer with oncogenic β-catenin signaling (119, 120). However, targeting Wnt/β-catenin signaling in HSCs to reduce ECM deposition in liver fibrosis may potentially be effective (186–188), but other studies indicate that it could potentially exacerbate fibrogenesis by promoting HSC activation (184, 185). The pleiotropic nature of this signaling pathway would also necessitate the development of delivery methods that would minimize off-target effects of undesirable Wnt/β-catenin signaling inhibition in nontarget cell types.

Despite all that is known about Wnt/β-catenin signaling in liver biology, clearly much still needs to be discovered. For example, very little is understood about which Wnt ligands interact with which Frizzled receptors in many cell types and disease contexts. This is a problem of almost dizzying complexity because there are 19 Wnts and 10 Frizzled receptors in the mammalian genome (22). Additionally, the role of noncanonical Wnt signaling in liver homeostasis, disease, and regeneration is poorly understood. Finally, the role of Wnt/β-catenin signaling in the pathobiology of conditions such as steatohepatitis (98, 103), cholestasis (48), and hepatic fibrosis (186, 187) in human patients will need to be investigated further, as experimental evidence has implicated a role for Wnt/β-catenin signaling in the etiology of these conditions. Undoubtedly, future research will continue to identify new and exciting roles for Wnt/β-catenin signaling in liver pathobiology and regeneration.

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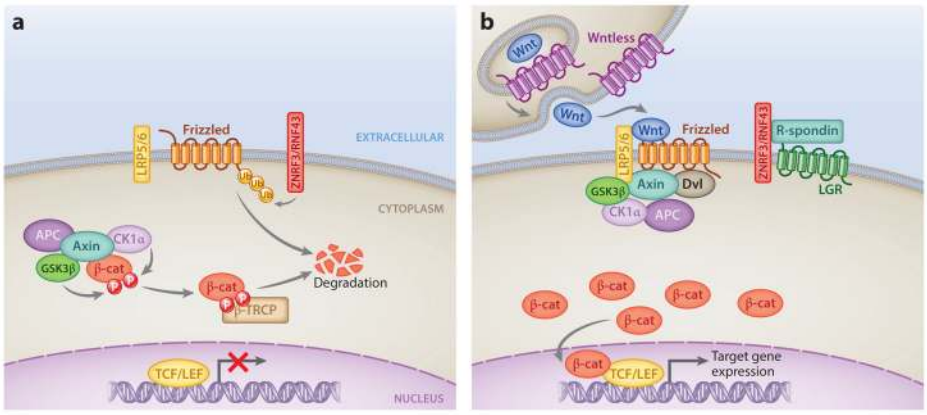

Figure 1.

The canonical Wnt signaling pathway. (*a*) In the absence of Wnt binding to its receptor (Frizzled) and coreceptor (LRP5/6), β-catenin is phosphorylated by its destruction complex and targeted for proteasomal degradation by βTRCP. The Frizzled receptor is targeted for proteasomal degradation via the activity of ZNRF3/RNF43. (*b*) Upon release of biologically active Wnt from a neighboring cell by cargo receptor Wntless, the Wnt protein binds its receptor and coreceptor, which triggers recruitment of the β-catenin destruction complex to the plasma membrane through scaffolding protein Dishevelled. This interaction is further stabilized by R-spondin binding to an LGR receptor. β-catenin cannot be phosphorylated, accumulates in the cytoplasm, and translocates to the nucleus to bind the TCF/LEF family of transcription factors to induce target gene transcription. Abbreviations: APC, adenomatous polyposis coli; β-cat, β-catenin; βTRCP, β-transducin repeat-containing protein; CK1α, casein kinase 1α; Dvl, Dishevelled; GSK3β, glycogen synthase kinase 3β; LGR, leucine-rich repeat-containing G protein–coupled receptor; LRP, lipoprotein receptor-related protein; RNF43, ring finger 43; TCF/LEF, T cell factor/lymphoid enhancer factor; ZNRF3, zinc and ring finger 3.

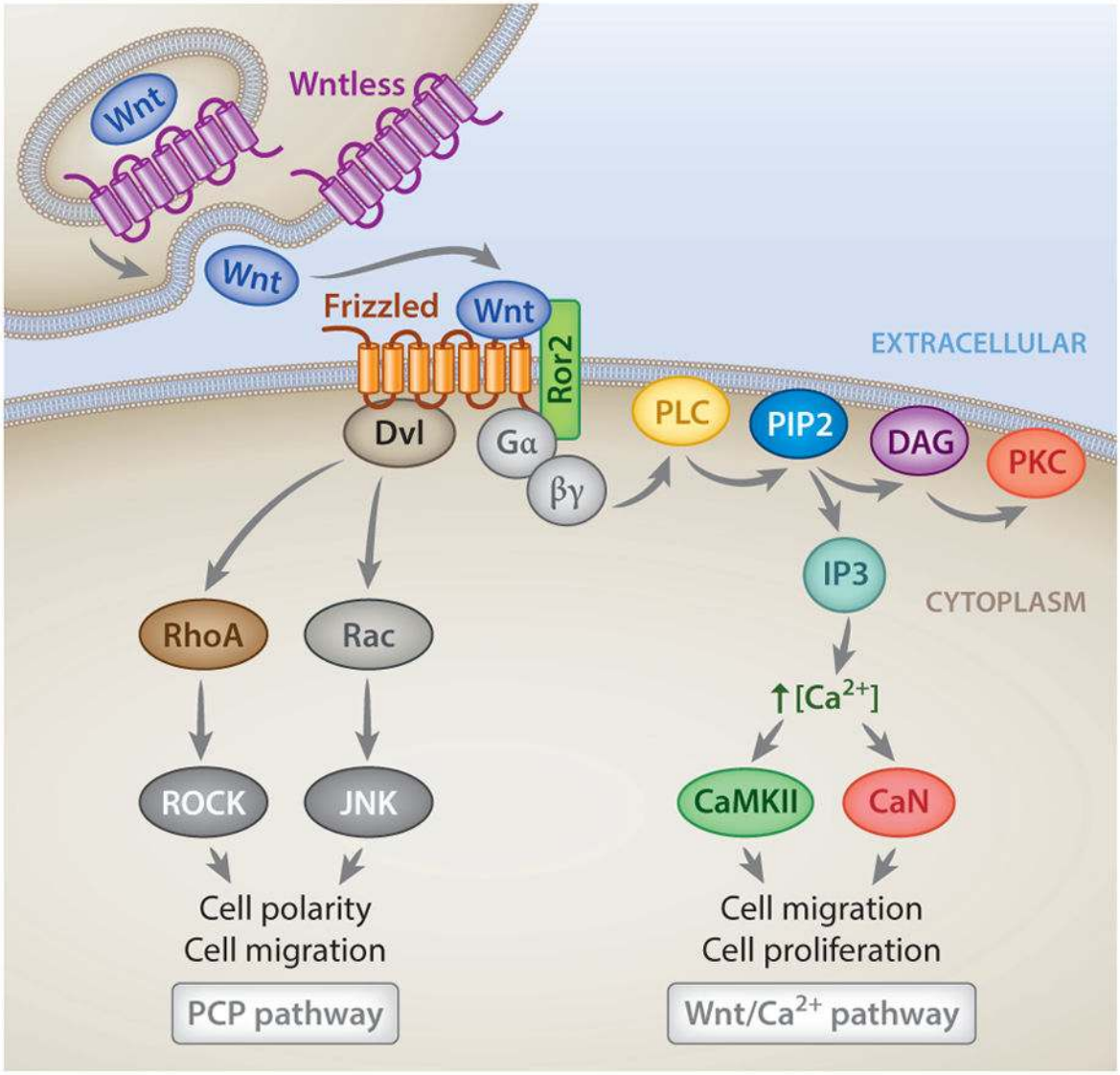

Figure 2.

Noncanonical Wnt signaling pathway. (*Left*) In the planar cell polarity pathway, Wnt ligands bind to a complex consisting of certain Frizzled receptors, Ror2, and Dishevelled, which triggers activation of RhoA and ROCK or, alternatively, activation of Rac and JNK signaling, to regulate cell polarity and migration. (*Right*) In the Wnt/calcium pathway, Wnt ligands bind to a complex consisting of Frizzled receptors, Dishevelled, and G proteins, which leads to the activation of PLC and the generation of DAG and IP3. DAG activates PKC whereas IP3 promotes increased intracellular calcium levels, which leads to the activation of CaMKII and CaN, which, in turn, regulates cell migration and proliferation. Abbreviations: βγ, G protein signaling subunit β/γ; CaMKII, calcium/calmodulin-dependent; CaN, calcineurin; DAG, diacylglycerol; Dvl, Dishevelled; Gα, G protein subunit α; IP3, inositol 1,4,5-triphosphate; JNK, c-Jun N-terminal kinase; PCP, planar cell polarity; PKC, protein kinase C; PLC, phospholipase C; PIP2, phosphatidylinositol 4,5 biphosphate;

Rac, Ras-related C3 botulinum toxin substrate; RhoA, Ras homolog gene family, member A; ROCK, Rho-associated protein kinase; Ror2, receptor tyrosine kinase-like orphan receptor 2.

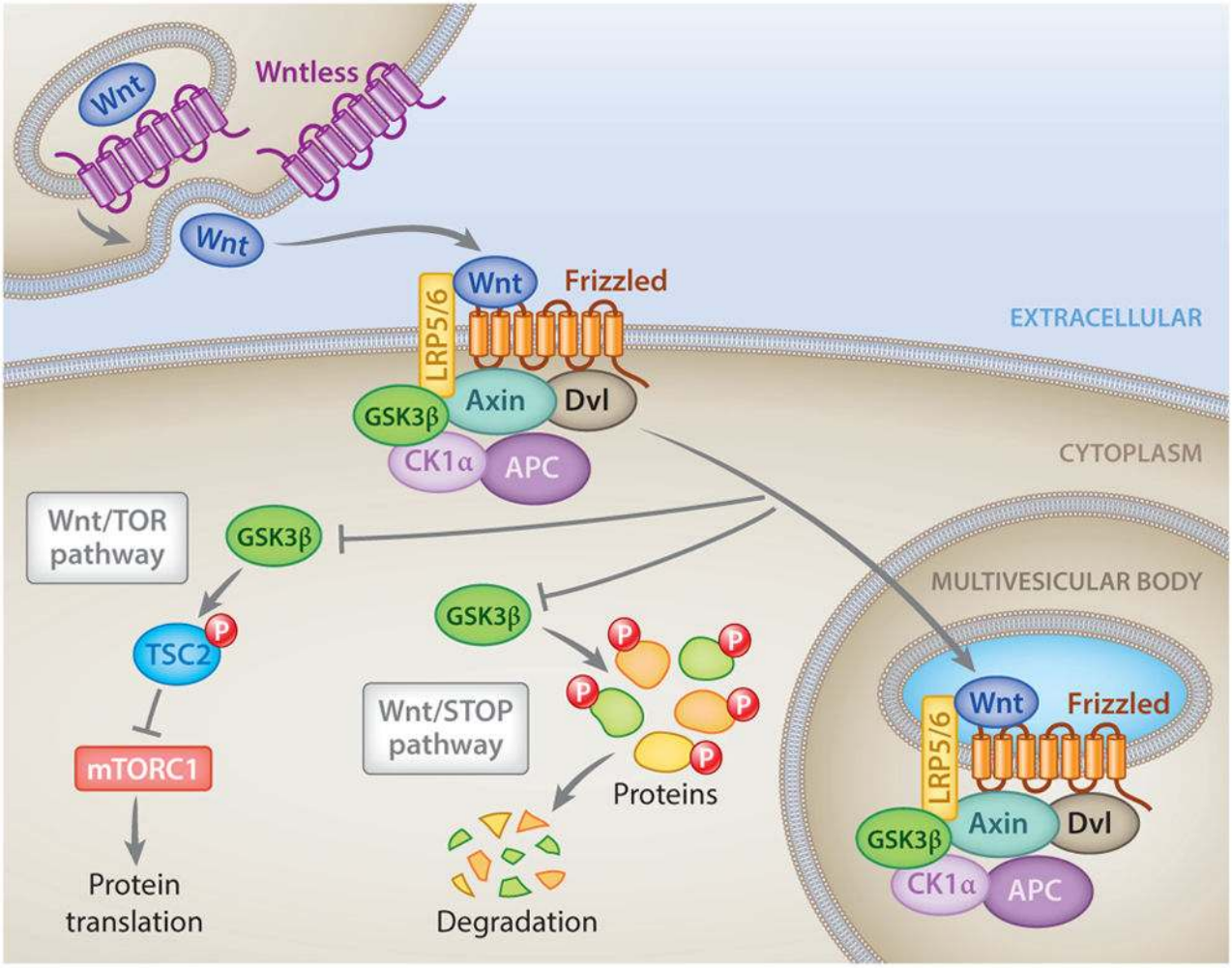

Figure 3.

Wnt/TOR and Wnt/STOP signaling pathways. (*Left*) In the Wnt/TOR pathway, in the absence of Wnt ligands, GSK3β phosphorylates and activates TSC2, which, in turn, inhibits mTORC1 activity. Binding of the Wnt protein to its receptor and coreceptor leads to sequestration of the destruction complex, including GSK3β, into multivesicular bodies. This prevents activation of TSC2, leading to activation of mTORC1 and promotion of protein translation. (*Right*) In the Wnt/STOP pathway, the activity of GSK3β promotes phosphorylation and proteasomal degradation of a multitude of target proteins. Upon Wnt ligand binding and sequestration of GSK3β into multivesicular bodies, these GSK3β-target proteins are no longer targeted for degradation and accumulate in the cytoplasm. Abbreviations: APC, adenomatous polyposis coli; CK1α, casein kinase 1α; Dvl, Dishevelled; GSK3β, glycogen synthase kinase 3β; LRP, lipoprotein receptor-related protein; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; TSC2, tuberous sclerosis complex 2; Wnt/STOP, Wnt-dependent stabilization of proteins; Wnt/TOR, Wnt-dependent regulation of mTOR.

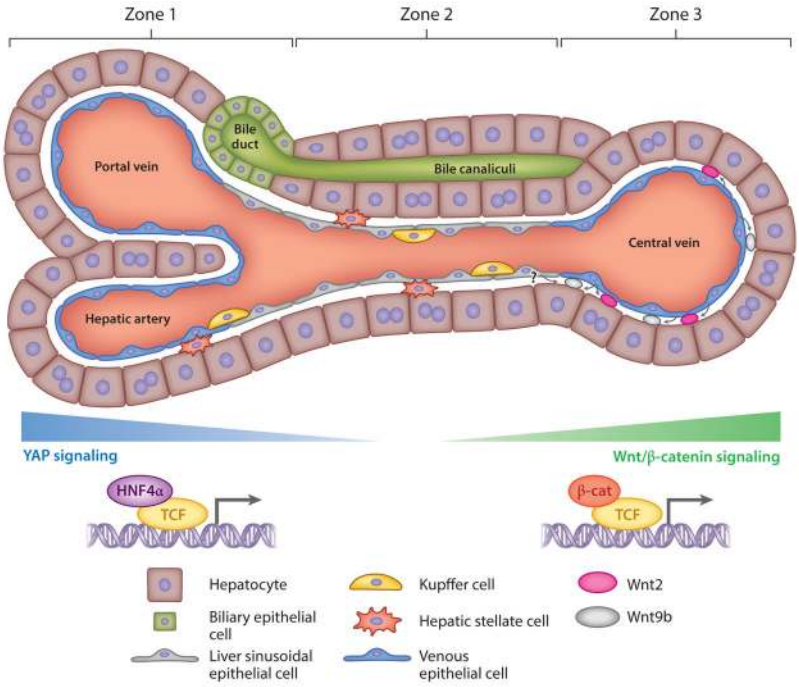

Figure 4.

Architecture of the hepatic sinusoid and metabolic zonation. The hepatic sinusoid is composed of multiple, carefully organized cell types. The main epithelial cells of the liver, the hepatocytes, are arranged in chords stretching from a portal triad, which consists of the portal vein, bile duct, and hepatic artery, to the central vein. These chords of hepatocytes are lined by specialized LSECs, which allow optimal perfusion of hepatocytes by the sinusoidal blood. Hepatic stellate cells reside in the space between hepatocytes and LSECs, whereas the tissue-resident macrophages, known as Kupffer cells, are adherent to LSECs within the hepatic sinusoid. The second epithelial cell type of the liver, biliary epithelial cells, lines the bile ducts in the portal triad. In terms of metabolic zonation, the hepatocytes adjacent to the portal triad constitute zone 1, where HNF4α binds to TCF to promote expression of the periportal gene signature. This zone is also characterized by periportal YAP signaling, which decreases in a gradient with increasing distance from the portal triad. The hepatocytes lining the central vein constitute zone 3, characterized by active Wnt/β-catenin driven via Wnt2 and Wnt9b secretion from venous endothelial cells. In this zone, β-catenin binds to TCF to promote expression of the pericentral gene signature. The hepatocytes in between zones 1 and 3 constitute zone 2, and display relatively low YAP and Wnt/β-catenin signaling. Abbreviations: β-cat, β-catenin; HNF4α, hepatocyte nuclear factor 4α; LSECs, liver sinusoidal endothelial cells; TCF, T cell factor; YAP, Yes-associated protein.

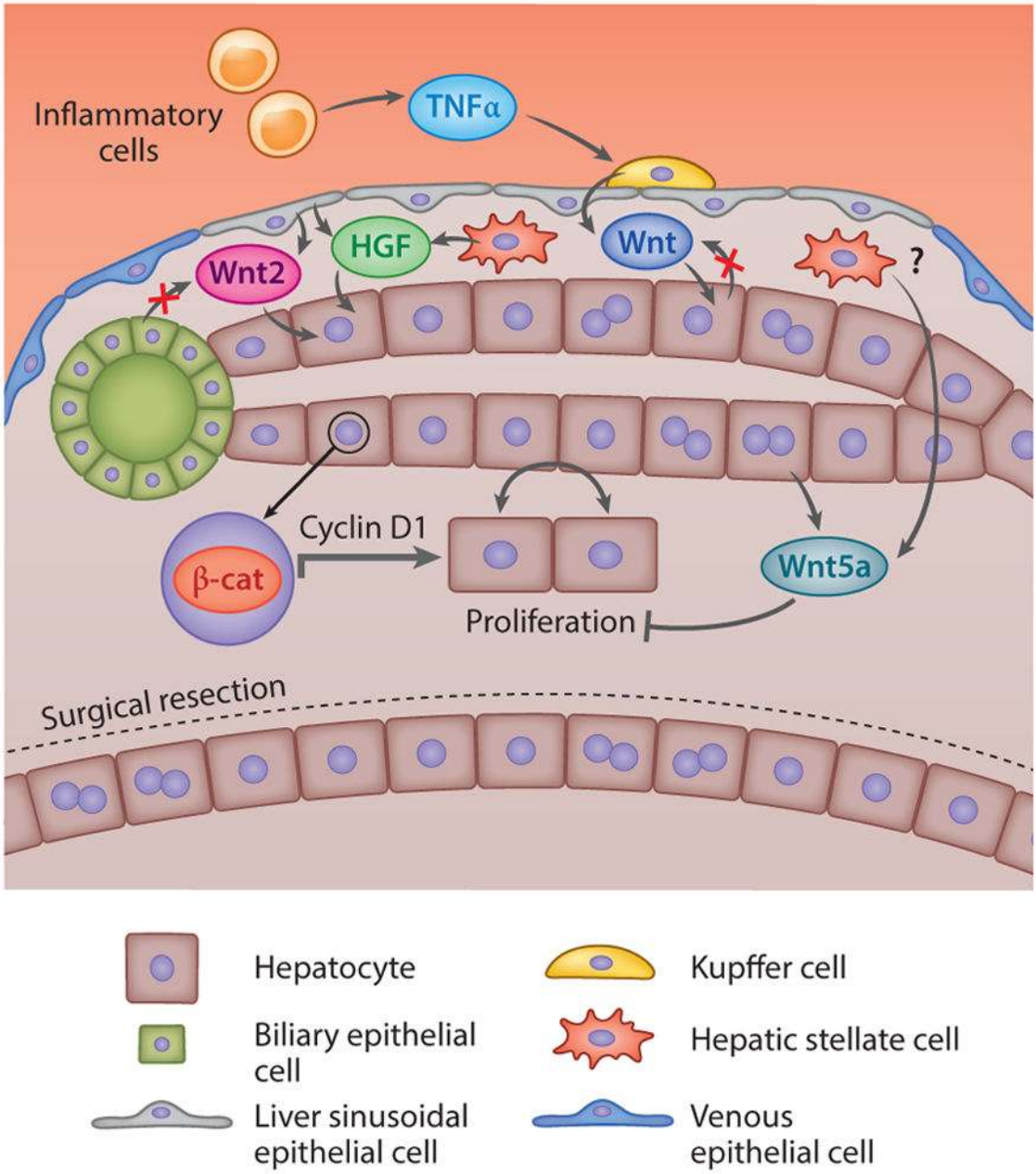

Figure 5.

Role of Wnt/β-catenin signaling in liver regeneration following surgical resection. Following a surgical resection of liver mass, Wnt/β-catenin signaling is activated to promote liver regeneration. Infiltrating inflammatory cells secrete TNFα, which promotes Wnt expression in macrophages. Additionally, liver sinusoidal endothelial cells secrete Wnt2 and HGF; the latter is also secreted by hepatic stellate cells. Neither biliary epithelial cells nor hepatocytes secrete mitogenic Wnts following surgical resection. The secreted Wnt ligands act on hepatocytes to promote β-catenin translocation to the nucleus, where it promotes expression of target genes, such as cyclin D1, to promote hepatocyte proliferation. Following the restoration of sufficient liver mass, hepatocytes secrete Wnt5a to inhibit canonical Wnt/β-

catenin signaling and promote termination of liver regeneration. Abbreviations: β-cat, β-catenin; HGF, hepatocyte growth factor; TNFα., tumor necrosis factor α.

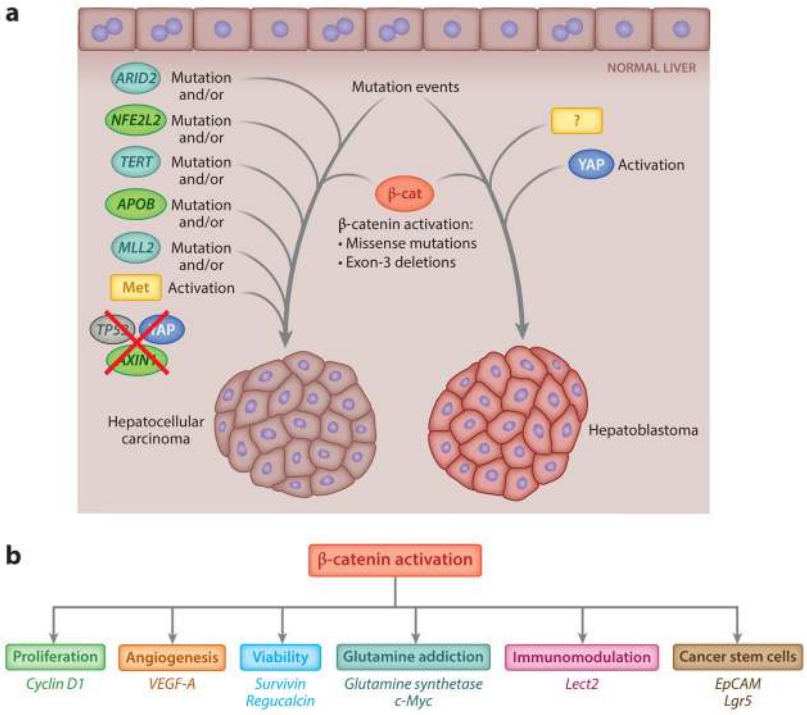

Figure 6.

Role of β-catenin in liver tumors. (*a*) Oncogenic activation of β-catenin occurs in multiple types of liver cancer, including HCC and HB. Common mechanisms of β-catenin activation include missense mutations and exon-3 deletions. In HCC, oncogenic β-catenin activation cooperates with mutations in proteins such as *ARID2*, *NFE2L2*, *TERT*, *APOB*, and *MLL2* or with Met activation to drive tumorigenesis. Interestingly, mutations in *TP53* and *AXIN1* or YAP activation tend to be mutually exclusive with β-catenin activation in HCC. In contrast, concomitant activation of β-catenin and YAP signaling is observed in approximately 80% of HB tumor samples, which suggests there are distinct mechanisms of hepatocarcinogenesis involving β-catenin and YAP in HB and HCC. (*b*) Wnt/β-catenin signaling activation regulates many aspects of tumor biology, as various downstream targets promote multiple oncogenic processes, including proliferation, survival, metabolism, immune tolerance, and angiogenesis. Abbreviations: EpCAM, epithelial cell adhesion molecule; HB, hepatoblastoma; HCC, hepatocellular carcinoma; Lect2, leukocyte cell–derived chemotaxin 2; Lgr5, leucine-rich repeat-containing G protein–coupled receptor 5; VEGF-A, vascular endothelial growth factor A; YAP, Yes-associated protein.

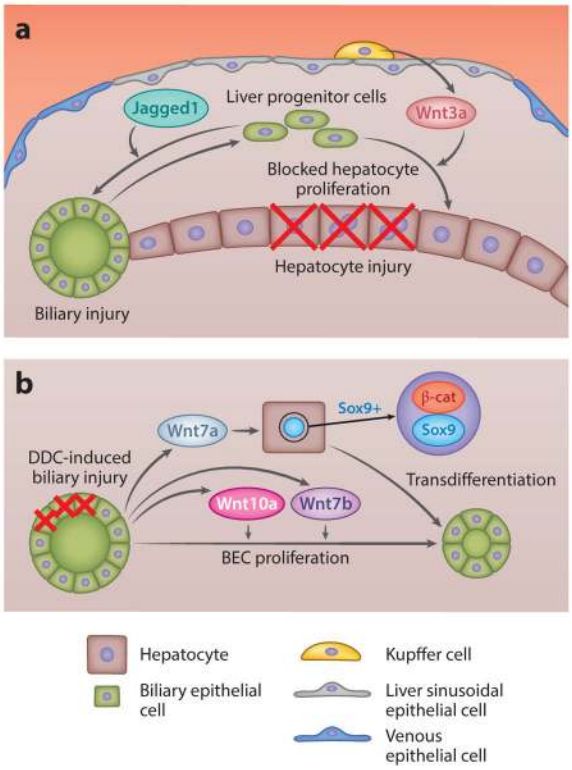

Figure 7.

Role of Wnt/β-catenin signaling in stem cell–mediated liver regeneration. (*a*) In conditions of extreme liver injury associated with a block of hepatocyte proliferation, bipotent liver progenitor cells (LPCs) arise from the biliary epithelial cell (BEC) compartment. Secretion of Wnt3a from Kupffer cells induces Wnt/β-catenin signaling in LPCs and promotes their transdifferentiation into hepatocytes to mediate regeneration from hepatocyte injury. Alternatively, induction of Notch signaling through Jagged1 promotes LPC transdifferentiation to BECs to mediate regeneration from biliary injury. (*b*) During conditions of biliary injury, such as that induced by a DDC diet, BECs secrete Wnt7a, which induces Sox9 expression and β-catenin activation in hepatocytes and promotes their transdifferentiation into BECs. Additionally, BECs secrete Wnt7b and Wnt10a to promote proliferation of BECs in a β-catenin-independent manner. Abbreviations: BEC, biliary epithelial cell; β-cat, β-catenin; DDC, hepatotoxin 3,5-diethoxycarbonyl-1, 4-dihydrocollidine; LPC, liver progenitor cell.