Minireview

Wnt signaling in biliary development, proliferation, and fibrosis

Lipeng Tian[1], Yichen Wang[1] and Yoon Young Jang[1,2]

[1]Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; [2]Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
Corresponding author: Yoon-Young Jang. Email: yjang3@jhmi.edu

Impact statement

Wnt signaling pathway has long been recognized to involve in the hepatogenesis and various liver damages. Understanding the mechanisms of Wnt pathway regulation in cholangiocyte generation and proliferation is important to elucidate the pathogenesis of biliary fibrosis. We summarized the diverse effects of Wnt signaling in the embryonic generation of hepatobiliary epithelial cells and the ductular reaction after liver ductal damage. Modulating Wnt pathway, especially the Wnt-PCP signaling, can be a potential strategy to reduce and/or reverse hepatobiliary fibrosis.

Abstract

Biliary fibrosis is an important pathological indicator of hepatobiliary damage. Cholangiocyte is the key cell type involved in this process. To reveal the pathogenesis of biliary fibrosis, it is essential to understand the normal development as well as the aberrant generation and proliferation of cholangiocytes. Numerous reports suggest that the Wnt signaling pathway is implicated in the physiological and pathological processes of cholangiocyte development and ductular reaction. In this review, we summarize the effects of Wnt pathway in cholangiocyte development from embryonic stem cells, as well as the underlying mechanisms of cholangiocyte responses to adult ductal damage. Wnt signaling pathway is regulated in a step-wise manner during each of the liver differentiation stages from embryonic stem cells to functional mature cholangiocytes. With the modulation of Wnt pathway, cholangiocytes can also be generated from adult liver progenitor cells and mature hepatocytes to repair liver damage. Non-canonical Wnt signaling is triggered in the active ductal cells during biliary fibrosis. Targeted control of the Wnt signaling may hold the great potential to reduce and/or reverse the biliary fibrogenic process.

Keywords: Pluripotent stem cells, Wnt signaling pathway, cholangiocyte, hepatobiliary development, biliary fibrosis, ductular reaction

Experimental Biology and Medicine **2022; 247: 360–367. DOI: 10.1177/15353702211061376**

Introduction

Biliary fibrosis is characterized by the abnormal deposition of insoluble extracellular matrix around the hepatobiliary ductal tissues. It usually occurs due to chronic injuries to the cholangiocyte along the biliary tree,[1] including chronic biliary obstruction, primary sclerosing cholangitis (PSC), and parasitic infection. It can also happen inherently due to defects of hepatobiliary duct development, such as biliary atresia.[2] When the causes of the disease are stopped, the fibrosis can regress. However, the excessive and chronic fibrosis following continuing fibrotic stimulation can cause the accumulation of cross-linked scar tissue, which cannot be completely reversed and remodeled.[3] Thus, there is a clinical need to examine the pathology of biliary fibrosis to develop the potential fibrosis-reversing agents, which can be used to reduce or prevent the excessive scarring.

The formation of biliary fibrosis requires the proliferation of cholangiocytes, activation of fibrogenic cells, and the deposition of insoluble extracellular matrix. The cholangiocyte lining the biliary duct can be generated from three sources before its proliferation in liver injury. The main source of cholangiocyte comes from embryonic development. However, cholangiocytes can also be differentiated from bipotent adult liver progenitor cells and transdifferentiated from hepatocytes.[4,5] Wnt signaling pathway has been demonstrated to play an essential role in regulating cholangiocyte development, proliferation, and fibrotic responses.[6] Here, we discuss the roles of the Wnt pathway during the embryonic development of cholangiocytes, adult proliferation of these cells, and biliary fibrosis process. Understanding the effects of the Wnt signaling pathway may bring forth the discovery of effective Wnt modulators to prevent and treat biliary fibrosis.

ISSN 1535-3702

Overview of Wnt signaling pathway

The Wnt signaling pathway is evolutionarily conserved. It can be classified into canonical and non-canonical pathways based on the involvement of β-catenin [7] (Figure 1 (a)). In the canonical pathway, Wnt ligands bind to Frizzled family receptors and low-density lipoprotein receptors (LRP) located on cells membrane, disrupt the β-catenin destruction complex, and lead to the accumulation of dephosphorylated β-catenin in the cytoplasm. Then, β-catenin translocates into the nucleus, where it interacts and activates the T cell factor/lymphoid enhancer factor-1 (TCF/Lef1) transcription complex to trigger the transcription of β-catenin-targeted genes (Figure 1(b)). In the absence of Wnt ligands, the β-catenin destruction complex, consisting of APC, Axin, CK1α, and GSK3β, phosphorylates β-catenin. The phosphorylated β-catenin in the cytoplasm subsequently undergoes ubiquitination and proteasome degradation.[8]

The non-canonical Wnt pathway does not depend on β-catenin. Several classic pathways of this group have been reported. In the Wnt/planar cell polarity (Wnt-PCP) pathway, Wnt ligands bind to Frizzled receptors and orphan receptor tyrosine kinases, such as protein tyrosine kinase 7 (PTK7) and receptor tyrosine kinase-like orphan receptor1/2 (ROR1/2). This complex activates scaffolding proteins Vang-like 1 (Vangl1) and Vang-like2 (Vangl2), which interact with the adapter protein Disheveled (Dsh). Dsh then further activates G protein Rho, which activates Rho-associated kinase (ROCK) to regulate cytoskeleton.[9] Dsh also binds to Rac1 and regulate cell polarity and migration through JNK-c-Jun[10] (Figure 1(c)). In the Wnt calcium non-canonical pathway, Wnt ligands bind to Frizzled receptors and activates phospholipase C. Phospholipase C further cleaves phosphatidylinositol 4,5 bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). The DAG in turn stimulates protein kinase C to increase intracellular calcium.

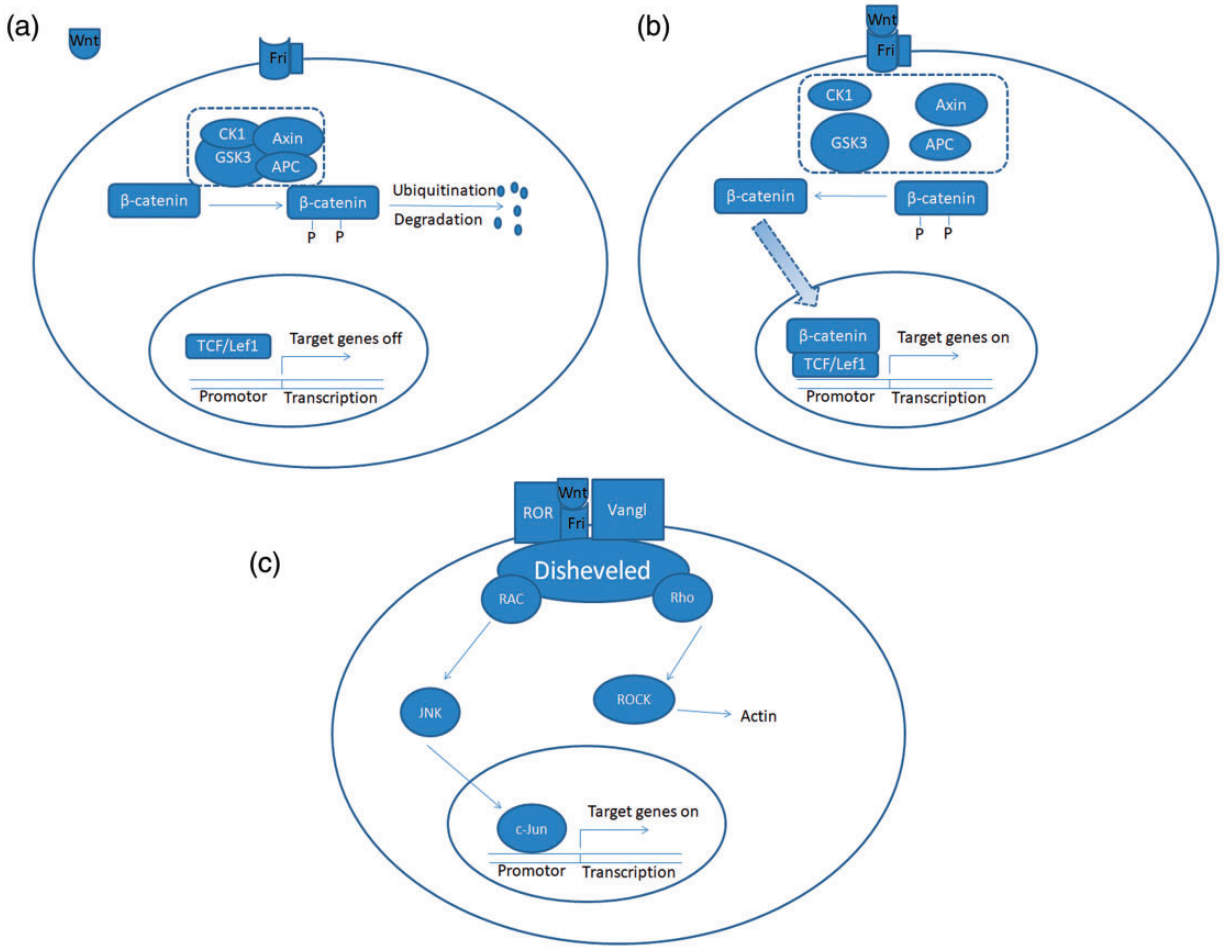

Figure 1. Overview of Wnt signaling pathway. (a) Illustration of OFF status of canonical Wnt pathway. Without Wnt ligands, β-catenin is phosphorylated by β-catenin destruction complex (dashed box). The phosphorylated β-catenin is further degraded through ubiquitination by proteasome. The target genes of Wnt pathway are inhibited. (b) Illustration of ON status canonical Wnt pathway. The binding of Wnt ligands to Frizzled family receptors disrupts the β-catenin destruction complex. Dephosphorylated β-catenin accumulates in the cytoplasm, which is further translocated into nucleus. The β-catenin interacts with TCF/Lef1 triggering Wnt target genes expression. (c) Illustration of non-canonical Wnt-PCP pathway. After Wnts bind to Frizzled receptors and co-receptors, Vangl interacts with Disheveled (Dsh). Dsh forms complex with Rac1 and mediates JNK-c-Jun pathway to regulate cell polarity. Dsh also activates G protein Rho, which further activates ROCK to regulate actin. (A color version of this figure is available in the online journal.)

Wnt pathway in embryonic biliary epithelial cell development

Hepatocyte and cholangiocyte are the two main types of epithelial cells in the liver, both of which are derived from the endoderm during embryonic differentiation. It is well acknowledged that the TGF-β family proteins play an essential role in regulating endoderm formation. Nodal, a member of TGF-β family, is expressed before gastrulation and initiates the formation of mesoderm and endoderm.[11] Activin A, another TGF-β family member, has also been demonstrated to induce the expression of endoderm markers including Sox17, CXCR4, and Foxa2 during the cholangiocyte differentiation from embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC).[12] It has been shown that the Wnt pathway actively participates in the gastrulation process.[13] The expressions of several Wnt ligands have been observed in the early primitive streak, such as Wnt3a[14] and Wnt5a.[15] Wnt3 knockout mice failed to produce primitive streak or primitive node, indicating that Wnt/β-catenin signaling is critical in the formation of endoderm and mesoderm.[16,17] Mice lacking β-catenin are embryonic lethal due to gastrulation defects.[18] More recent studies suggest that pre-exposure to the Wnt signaling pathway can potentiate the response to TGF-β signals in human stem cells (Figure 2).[19] It is also indicated that early activations of the Wnt pathway by CHIR99021, a GSK3β inhibitor, promote endoderm differentiation in embryonic stem cells and iPSCs.[20] The mechanism of such methods may be partly explained by the localization of β-catenin/Tcf transactivation complexes on Sox17 cis-regulatory elements.[21]

During early somitogenesis, the endoderm cells form an epithelial gut tube, which further forms three different domains along the anterior-posterior axis: foregut, midgut, and hindgut. The cholangiocyte and hepatocyte are derived from the foregut. Recent evidence suggests that repression of Wnt and FGF4 signaling is required to pattern endoderm into foregut (Figure 2).[22,23] In Xenopus embryo, inhibition of the Wnt-β-catenin pathway in the anterior endoderm is necessary for foregut development. Highly expressed β-catenin in the posterior endoderm can inhibit foregut formation.[22] Secreted frizzled-related protein 5 (Sfrp5), a Wnt pathway antagonist, has been demonstrated to express in foregut epithelium, and the depletion of Sfrp5 leads to reduced expressions of foregut genes.[24] Furthermore, the experimental inhibition of Wnt-β catenin pathway in the posterior endoderm of Xenopus has shown to induce ectopic liver buds in the intestine.[22] The use of Wnt inhibition has been adopted into protocols directing iPSC and ESC towards the hepatic fate.[25]

While Wnt inhibition is essential for the foregut development, Wnt activation can promote hepatic fate from the foregut endoderm. It has been demonstrated that Wnt signaling is involved in the expression of many key hepatic fate-determining factors including HNF-1b, Foxa1, foxa2, and GATA4 during liver development.[26] Wnt2 in the lateral mesoderm has been implicated in the initiation of hepatic specification in zebrafish embryos, and defects of liver development were observed in the Wnt2bb and Wnt2 lacking zebrafish.[27] Wnt2 and Wnt2b are expressed in lateral mesoderm at the same time of hepatic induction in mice.[28] Additionally, Wnt8a overexpression induces expression of hepatic genes in the pancreatic and intestinal bulb cells.[27] These studies indicate the involvement of Wnt pathway activation in the hepatic fate determination and cell survival.

Shortly after hepatic fate determination, the liver diverticulum cells delaminate the surrounding laminin-rich basal layer, proliferate, and migrate into septum transversum mesenchyme (STM) to pattern the liver bud. STM secrets various factors, including FGF, HGF, BMPs, TGF-β, and RA to enhance hepatoblast proliferation. Hepatoblast migration and proliferation in STM also require interactions with the embryonic sinusoidal wall.[29] The endothelial cells in the sinusoidal wall express Wnt9a, which promotes the proliferation of hepatoblast. Overexpression of Wnt9a results in hepatomegaly, while knockdown of this gene could lead to a reduction of liver size.[29] An extensive cross-talking between the signals from STM and the sinusoidal wall appears to be involved. FGF and HGF signaling can induce a variety of intracellular signaling kinase cascades (MAPK, JNK, Pi3K) and promote the activity of β-catenin in the liver bud.[30]

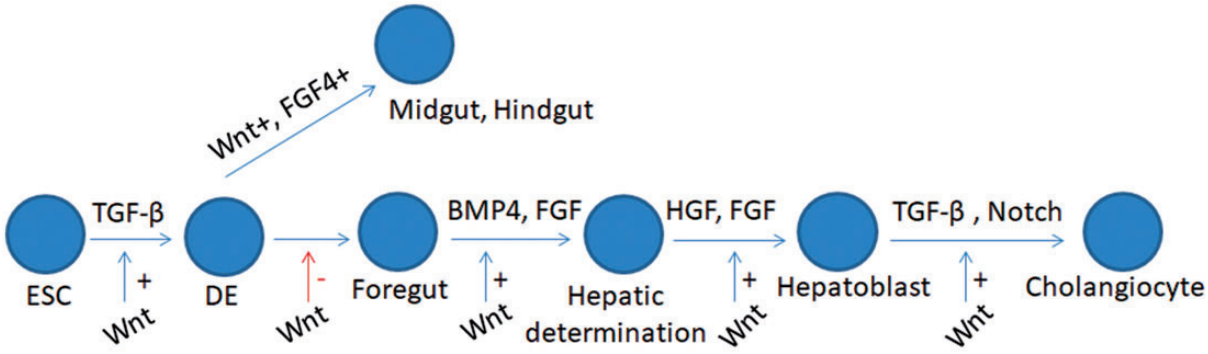

Figure 2. Illustration of the effects of Wnt pathway in embryonic biliary epithelial cell development. Wnt pathway is precisely regulated in every development stage from embryonic stem cells to functional mature cholangiocytes, biliary epithelial cells. Wnt activation can potentiate the definitive endoderm (DE) differentiation from ESC, while it must be inhibited to pattern the DE into foregut fate. However, Wnt activation determines the hepatic fate from foregut along with BMP4 and FGF signaling. Wnt pathway is also important for the proliferation and survival of hepatoblasts. Wnt activation plays a critical role in the differentiation of cholangiocytes from the hepatoblasts. (A color version of this figure is available in the online journal.)

The hepatoblasts are bi-potential, which can differentiate into hepatocytes or biliary cholangiocytes. These bipotent progenitors can differentiate into hepatocytes when they are not in contact with portal veins, while they adopt the biliary epithelium fate when located near the portal vein mesenchyme. The periportal mesenchyme cells secret TGF-β and Notch to stimulate ductal cell differentiation from hepatoblast. Blocking TGF-β signaling after hepatic specification dramatically reduces cholangiocyte formation.[31] The portal mesenchyme cells express Jagged 1, which can activate Notch2 in the adjacent hepatoblast to regulate ductal cells development.[32] Knockout of Notch2 in hepatoblasts can completely block the induction of bile duct formation.[33] It has also been shown that Wnt signaling promotes ductal development in this stage. Specifically, Wnt3a-conditioned media promotes the differentiation of hepatoblast into CK19-positive biliary cells.[34] The deletion of APC in hepatoblast, which results in β-catenin accumulation, promoted the biliary differentiation and impeded the hepatocyte fate determination.[35] Loss of β-catenin with a foxa3 promoter in hepatoblast also compromised the development of bile duct.[36] However, Wnt signaling may affect liver ductal cells development at an early stage. Liver ductal cells' maturation and morphogenesis appeared to be normal when β-catenin was deleted in ductal progenitors using SOX9-driven Cre recombinase.[37] These studies demonstrate that Wnt/β-catenin pathway must be precisely regulated throughout different stages of liver duct developments.

Wnt pathway in cholangiocyte generation from adult hepatic progenitor cells and hepatocytes

Biliary cholangiocyte plays an essential role in the transport of toxic bile acid. They are also known to act as a major cell source for regeneration after liver injury. Moreover, recent tracing studies have shown that biliary cells can function as hepatic progenitor cells (HPC) to generate hepatocytes in chronic liver injuries.[38,39] Meanwhile, cholangiocytes can also be regenerated from adult HPC and hepatocytes. After liver injury, adult HPCs are activated to regenerate cholangiocytes and hepatocytes to mediate liver regeneration. Adult HPCs have been believed to be derived from the canal of Hering,[40] the hepatocytic–biliary interface of bile canaliculi and intralobular bile duct (Figure 3(a)). Active β-catenin in the nucleus of HPC was observed in mice fed with 3,5-diethoxycarbonyl-1,4 dihydrocollidine (DDC) diet, which is an animal model mimicking sclerosing cholangitis,[41] suggesting canonical Wnt signaling in HPC was activated following bile duct injury (Figure 3(b)). Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is one of the Wnt target genes. The expression of LGR5 is not found in healthy adults. However, LGR5-positive HPC was observed near bile ducts in CCl4 or DDC-injured mice, and these cells can differentiate into hepatocytes and ductal cells *in vivo*.[42] Notably, LGR5 is a receptor of Wnt agonist R-spondin1. In choline-deficient and ethionine-supplemented (CDE) fed mice, expression of Jagged 1 on

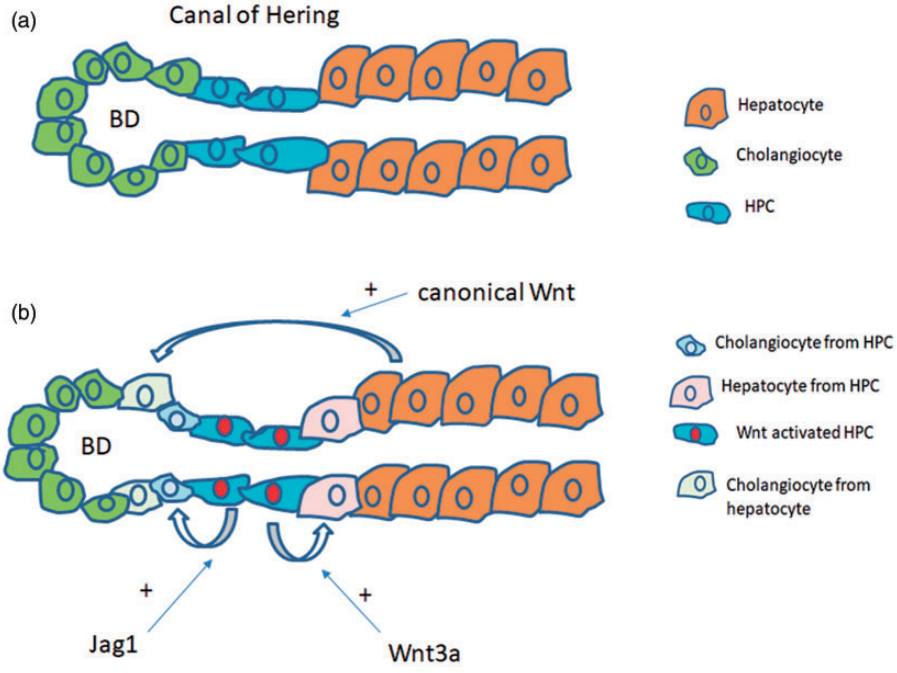

Figure 3. Effects of Wnt pathway in cholangiocyte generation from adult hepatic progenitor cells (HPC) and hepatocytes. (a) A diagram of canal of Hering. HPCs are located in the canal of Hering. (b) During ductal damage, HPC is activated with β-catenin in the nucleus. Jag1 from the myofibroblast directs the differentiation of HPC to cholangiocytes. Wnt3a from macrophage induces HPC to hepatocyte. Hepatocyte can transdifferentiate into cholangiocytes under canonical Wnt signaling stimulation. (A color version of this figure is available in the online journal.)

the myofibroblast specified HPC fate towards cholangiocyte, while Wnt3a expressing macrophage induced canonical Wnt pathway and directed HPC towards hepatocytes,[43] which suggest Notch signaling directs cholangiocyte generation from HPC, while canonical Wnt pathway may inhibit it (Figure 3(b)).

Hepatocyte and cholangiocyte have been proposed to function as facultative stem cells, which can generate each other in conditions of injury.[44] In lineage tracing study, hepatocytes can transdifferentiate into ductal cells in DDC-fed mice.[45] Dipeptidyl peptidase IV (DPPIV)-positive hepatocytes were injected into DPPIV-negative rats, after partial hepatectomy, to form the chimeric liver. After ligating the bile duct, the rats with chimeric livers showed donor DPPIV-positive biliary epithelial cells and formed ductules.[46] The hepatocytes expressing S45D mutated non-degradable β-catenin in the periportal region significantly express cholangiocyte markers including EpCAM, CK19, and Sox9 in DDC-fed mice,[6] suggesting a possibility that the activation of canonical Wnt pathway can induce conversion from hepatocytes to cholangiocytes.

Wnt pathway in biliary fibrosis and ductular reaction

The biliary fibrotic microenvironment is mainly composed of activated myofibroblasts, macrophages, and biliary epithelial cells. The proliferation of cholangiocytes, called ductular reaction, parallels with fibrosis in this niche. Wnt ligands are considered to be one of the most effective components in the microenvironment (Figure 4). The biliary fibrosis and ductular proliferation following ductal damage can be regarded as a repair process to maintain the architecture of the tissue. The chronic fibrogenic process can lead to excessive and irreversible scar deposition. Inhibiting Wnt ligands secretion by conditioned Wntless knockout in the liver can decrease ductal proliferation and increase mortality in DDC-fed mice.[6] Wnt ligands proteins were upregulated in the portal triad of DDC-fed mice, and the expression of Wntless was observed in EpCAM-positive cholangiocytes and macrophages.[6] This indicates that cholangiocytes and macrophages are two main Wnts sources in the fibrotic niche.

Macrophages have been realized as an important Wnt producing source.[47,48] They are among the first responders to biliary injury. Macrophages can upregulate the expression of Wnt ligands through phagocytosis of dead or dying cells. Expressions of Wnt11 are up-regulated in macrophages of mice on a DDC diet.[48] The expressions of Wnt5a mRNA and protein were increased in CD68+ macrophages located near biliary epithelial cells.[49] The bile duct tissue showed reduced CTGF expression and collagen 1 immunostaining when Wnt5a was deleted from macrophages. On the contrary, increased EpCAM expression, HPC activation, and fibrosis, along with ductular reaction are observed when Wntless is deleted in myeloid cells in the thioacetamide (TAA) model.[50] The expression of Timp1, a key matrix metalloproteinase (MMP) inhibitor to block collagen degradation, is increased in this model, suggesting macrophage-derived Wnt proteins restraining fibrosis and ductular reaction. These contradictory findings further demonstrated the complexity of Wnt proteins derived from macrophages.

Biliary epithelial cells have a remarkable regeneration capability following liver injury. Axin2 expression is recognized as a marker of canonical Wnt pathway activation. Axin2 and LGR5 are expressed in hepatocytes but not in cholangiocytes during the ductal reaction, and mice with conditioned knockout of LGR4 and LGR5 in EpCAM-

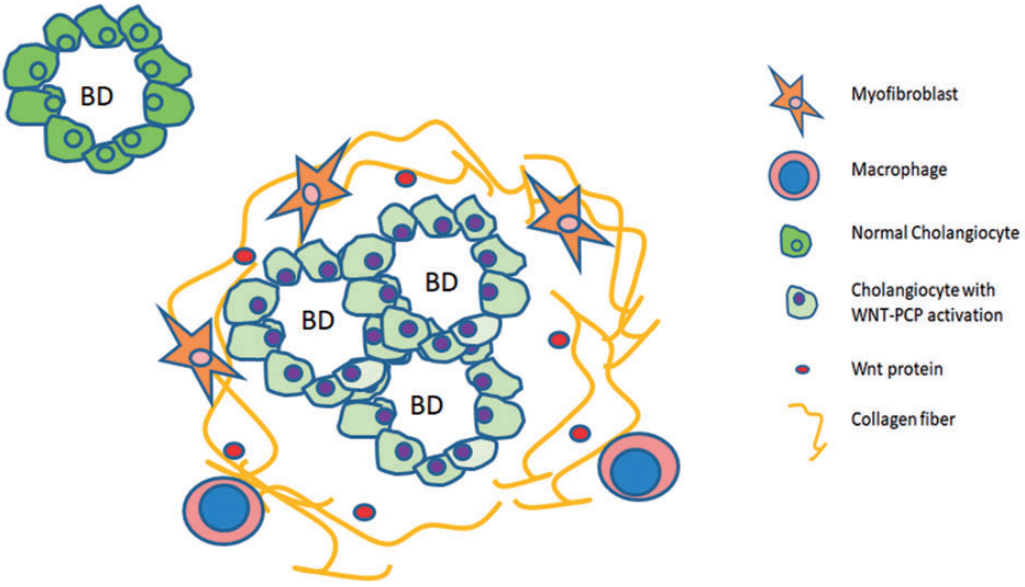

Figure 4. Biliary fibrotic microenvironment. The biliary fibrotic niche is mainly composed by activated myofibroblasts, macrophages, and biliary epithelial cells. Wnt proteins are the main effective components in this niche. Wnt-PCP pathway is activated in ductal cells during biliary fibrosis. The canonical Wnt β-catenin pathways are not observed. Cholangiocytes and macrophages are the two important Wnt sources. (A color version of this figure is available in the online journal.)
BD: bile ducts.

positive cells failed to show changes of ductal reaction.[51] It suggests that the canonical Wnt β-catenin pathway has a limited role in ductal proliferation. Furthermore, the accumulation of β-catenin in the nucleus was not detected in tissue from patients with primary sclerosing cholangitis.[49] Additionally, the canonical Wnt pathway is also dispensable during *in vitro* culture of cholangiocyte organoids.[52]

Recent single-cell RNA sequencing indicated that Wnt7a, Wnt7b, and Wnt10a were abundantly upregulated in biliary epithelial cells in DDC-fed mice,[53] without LGR5 or Axin2 induction, suggesting non-canonical Wnt-PCP signaling may control the activation of ductal reaction and fibrosis. Vangl2 and PTK7 proteins were found to localize on proliferating ductal cells in PSC patient tissues, but not in the healthy bile duct. It suggests the activation of Wnt-PCP pathway during the ductal reaction in PSC patients' livers (Figure 4).[49] In addition, a significantly increased expression of phosphorylated JNK and c-Jun is observed in ductal cells of the tissue from PSC patients and DDC-fed mice.[49] LGK974, a porcupine inhibitor to inhibit lipid modification to Wnt ligands, was shown to reduce p-JNK and p-c-Jun in the hepatic ducal cells both in TAA and DDC models,[49] suggesting that the activation of JNK/c-Jun is controlled by Wnt-PCP pathway.

Numerous reports have indicated that Wnt-PCP pathway can regulate extracellular matrix deposition.[54] Total fibrillar collagen deposition during the bile duct reaction was significantly reduced after inhibiting porcupine in DDC and TAA models.[49] Vangl2 mutation can reduce the deposition of extracellular matrix through increased MMP14 activity.[55] MMP14, also called membrane type 1 matrix metalloproteinase, acts as an activator of MMP2 and MMP9 to regulate fibrosis.[56] In DDC or TAA models, mice with Vangl2 heterozygous mutation showed significantly reduced ductular fibrosis, indicating Wnt-PCP signaling is essential for biliary scars. Emerging evidence from other diseases also indicates that Wnt-PCP pathway plays a significant role in the accumulation of extracellular matrix and the establishment of fibrosis.[57,58]

Conclusions

With increasing studies on the Wnt signaling pathway in hepatobiliary development and proliferation, it becomes clear that the Wnt pathway has major implication in a wide variety of pathophysiologic processes that are associated with cholangiocytes, from embryonic development to ductal reaction in adults. The complexity of Wnt interactions in the canonical and non-canonical downstream made it challenging to elucidate its roles during the ductular reaction. Wnt pathway can also interact with numerous other pathways to drive the cells towards or away from fibrosis. Notch and Yap signaling were also involved in ductal development and proliferation and biliary fibrosis.[43,51,53] This review described the diverse roles of the Wnt pathway in cholangiocyte development, proliferation, and biliary fibrosis. Turning on and off of the Wnt signaling in the correct temporal manner may be critical for differentiating embryonic stem cells and iPSC towards hepatocytes and cholangiocytes. Modulation of the Wnt pathway, especially the Wnt-PCP signaling, may hold the potential to reduce or reverse biliary fibrosis. Future research is warranted to discover novel and exciting roles of the Wnt signaling pathway in hepatobiliary development and pathophysiology.

AUTHORS' CONTRIBUTIONS

All authors participated in the review of the article. LT wrote the article and prepared the figures. YW contributed editing. YJ guided the article and figures, and made corrections.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the authorship, and publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and publication of this article: This work was supported by NIH grant R01 DK122982 and PSC Partners Research Grant, Johns Hopkins Pediatric Liver Center.

ORCID iD

Yoon Young Jang https://orcid.org/0000-0001-5462-2399

REFERENCES

1. Pinzani M, Luong TV. Pathogenesis of biliary fibrosis. *Biochim Biophys Acta Mol Basis Dis* 2018;**1864**:1279–83
2. Lakshminarayanan B, Davenport M. Biliary atresia: a comprehensive review. *J Autoimmun* 2016;**73**:1–9
3. Ramachandran P, Iredale JP. Liver fibrosis: a bidirectional model of fibrogenesis and resolution. *Qjm* 2012;**105**:813–7
4. Kitade M, Factor VM, Andersen JB, Tomokuni A, Kaji K, Akita H, Holczbauer A, Seo D, Marquardt JU, Conner EA, Lee SB, Lee YH, Thorgeirsson SS. Specific fate decisions in adult hepatic progenitor cells driven by MET and EGFR signaling. *Genes Dev* 2013;**27**:1706–17
5. Schaub JR, Huppert KA, Kurial SNT, Hsu BY, Cast AE, Donnelly B, Karns RA, Chen F, Rezvani M, Luu HY, Mattis AN, Rougemont AL, Rosenthal P, Huppert SS, Willenbring H. De novo formation of the biliary system by TGFbeta-mediated hepatocyte transdifferentiation. *Nature* 2018;**557**:247–51
6. Okabe H, Yang J, Sylakowski K, Yovchev M, Miyagawa Y, Nagarajan S, Chikina M, Thompson M, Oertel M, Baba H, Monga SP, Nejak-Bowen KN. Wnt signaling regulates hepatobiliary repair following cholestatic liver injury in mice. *Hepatology* 2016;**64**:1652–66
7. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell* 2006;**127**:469–80
8. Kimelman D, Xu W. Beta-catenin destruction complex: insights and questions from a structural perspective. *Oncogene* 2006;**25**:7482–91
9. Wang Y. Wnt/planar cell polarity signaling: a new paradigm for cancer therapy. *Mol Cancer Ther* 2009;**8**:2103–9
10. Habas R, Dawid IB, He X. Coactivation of Rac and Rho by Wnt/frizzled signaling is required for vertebrate gastrulation. *Genes Dev* 2003;**17**:295–309
11. Agius E, Oelgeschlager M, Wessely O, Kemp C, De Robertis EM. Endodermal nodal-related signals and mesoderm induction in xenopus. *Development* 2000;**127**:1173–83
12. Tian L, Ye Z, Kafka K, Stewart D, Anders R, Schwarz KB, Jang YY. Biliary atresia relevant human induced pluripotent stem cells recapitulate key disease features in a dish. *J Pediatr Gastroenterol Nutr* 2019;**68**:56–63

13. Wang J, Sinha T, Wynshaw-Boris A. Wnt signaling in mammalian development: lessons from mouse genetics. *Cold Spring Harb Perspect Biol* 2012;**4**:a007963
14. Takada S, Stark KL, Shea MJ, Vassileva G, McMahon JA, McMahon AP. Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev* 1994;**8**:174–89
15. Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* 1999;**126**:1211–23
16. Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A. Requirement for Wnt3 in vertebrate axis formation. *Nat Genet* 1999;**22**:361–5
17. Galceran J, Farinas I, Depew MJ, Clevers H, Grosschedl R. Wnt3a-/–like phenotype and limb deficiency in Lef1(-/-)Tcf1(-/-) mice. *Genes Dev* 1999;**13**:709–17
18. Haegel H, Larue L, Ohsugi M, Fedorov L, Herrenknecht K, Kemler R. Lack of beta-catenin affects mouse development at gastrulation. *Development* 1995;**121**:3529–37
19. Yoney A, Etoc F, Ruzo A, Carroll T, Metzger JJ, Martyn I, Li S, Kirst C, Siggia ED, Brivanlou AH. WNT signaling memory is required for ACTIVIN to function as a morphogen in human gastruloids. *Elife* 2018;**7**:e38279
20. Choi SM, Kim Y, Shim JS, Park JT, Wang RH, Leach SD, Liu JO, Deng C, Ye Z, Jang YY. Efficient drug screening and gene correction for treating liver disease using patient-specific stem cells. *Hepatology* 2013;**57**:2458–68
21. Engert S, Burtscher I, Liao WP, Dulev S, Schotta G, Lickert H. Wnt/beta-catenin signalling regulates Sox17 expression and is essential for organizer and endoderm formation in the mouse. *Development* 2013;**140**:3128–38
22. McLin VA, Rankin SA, Zorn AM. Repression of wnt/beta-catenin signaling in the anterior endoderm is essential for liver and pancreas development. *Development* 2007;**134**:2207–17
23. Dessimoz J, Opoka R, Kordich JJ, Grapin-Botton A, Wells JM. FGF signaling is necessary for establishing gut tube domains along the anterior-posterior axis in vivo. *Mech Dev* 2006;**123**:42–55
24. Li Y, Rankin SA, Sinner D, Kenny AP, Krieg PA, Zorn AM. Sfrp5 coordinates foregut specification and morphogenesis by antagonizing both canonical and noncanonical Wnt11 signaling. *Genes Dev* 2008;**22**:3050–63
25. Touboul T, Chen S, To CC, Mora-Castilla S, Sabatini K, Tukey RH, Laurent LC. Stage-specific regulation of the WNT/beta-catenin pathway enhances differentiation of hESCs into hepatocytes. *J Hepatol* 2016;**64**:1315–26
26. Lade AG, Monga SP. Beta-catenin signaling in hepatic development and progenitors: which way does the WNT blow? *Dev Dyn* 2011;**240**:486–500
27. Shin D, Lee Y, Poss KD, Stainier DY. Restriction of hepatic competence by fgf signaling. *Development* 2011;**138**:1339–48
28. Goss AM, Tian Y, Tsukiyama T, Cohen ED, Zhou D, Lu MM, Yamaguchi TP, Morrisey EE. Wnt2/2b and beta-catenin signaling are necessary and sufficient to specify lung progenitors in the foregut. *Dev Cell* 2009;**17**:290–8
29. Matsumoto K, Miki R, Nakayama M, Tatsumi N, Yokouchi Y. Wnt9a secreted from the walls of hepatic sinusoids is essential for morphogenesis, proliferation, and glycogen accumulation of chick hepatic epithelium. *Dev Biol* 2008;**319**:234–47
30. Shin D, Monga SP. Cellular and molecular basis of liver development. *Compr Physiol* 2013;**3**:799–815
31. Wang W, Feng Y, Aimaiti Y, Jin X, Mao X, Li D. TGFbeta signaling controls intrahepatic bile duct development may through regulating the Jagged1-Notch-Sox9 signaling axis. *J Cell Physiol* 2018;**233**:5780–91
32. Hofmann JJ, Zovein AC, Koh H, Radtke F, Weinmaster G, Iruela-Arispe ML. Jagged1 in the portal vein mesenchyme regulates intrahepatic bile duct development: insights into alagille syndrome. *Development* 2010;**137**:4061–72
33. Falix FA, Weeda VB, Labruyere WT, Poncy A, de Waart DR, Hakvoort TB, Lemaigre F, Gaemers IC, Aronson DC, Lamers WH. Hepatic Notch2 deficiency leads to bile duct agenesis perinatally and secondary bile duct formation after weaning. *Dev Biol* 2014;**396**:201–13
34. Hussain SZ, Sneddon T, Tan X, Micsenyi A, Michalopoulos GK, Monga SP. Wnt impacts growth and differentiation in ex vivo liver development. *Exp Cell Res* 2004;**292**:157–69
35. Decaens T, Godard C, de Reynies A, Rickman DS, Tronche F, Couty JP, Perret C, Colnot S. Stabilization of beta-catenin affects mouse embryonic liver growth and hepatoblast fate. *Hepatology* 2008;**47**:247–58
36. Tan X, Yuan Y, Zeng G, Apte U, Thompson MD, Cieply B, Stolz DB, Michalopoulos GK, Kaestner KH, Monga SP. Beta-catenin deletion in hepatoblasts disrupts hepatic morphogenesis and survival during mouse development. *Hepatology* 2008;**47**:1667–79
37. Cordi S, Godard C, Saandi T, Jacquemin P, Monga SP, Colnot S, Lemaigre FP. Role of beta-catenin in development of bile ducts. *Differentiation* 2016;**91**:42–9
38. Deng X, Zhang X, Li W, Feng RX, Li L, Yi GR, Zhang XN, Yin C, Yu HY, Zhang JP, Lu B, Hui L, Xie WF. Chronic liver injury induces conversion of biliary epithelial cells into hepatocytes. *Cell Stem Cell* 2018;**23**:114–22 e3
39. Raven A, Lu WY, Man TY, Ferreira-Gonzalez S, O'Duibhir E, Dwyer BJ, Thomson JP, Meehan RR, Bogorad R, Koteliansky V, Kotelevtsev Y, Ffrench-Constant C, Boulter L, Forbes SJ. Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. *Nature* 2017;**547**:350–4
40. Kordes C, Haussinger D. Hepatic stem cell niches. *J Clin Invest* 2013;**123**:1874–80
41. Hu M, Kurobe M, Jeong YJ, Fuerer C, Ghole S, Nusse R, Sylvester KG. Wnt/beta-catenin signaling in murine hepatic transit amplifying progenitor cells. *Gastroenterology* 2007;**133**:1579–91
42. Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, Sato T, Hamer K, Sasaki N, Finegold MJ, Haft A, Vries RG, Grompe M, Clevers H. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature* 2013;**494**:247–50
43. Boulter L, Govaere O, Bird TG, Radulescu S, Ramachandran P, Pellicoro A, Ridgway RA, Seo SS, Spee B, Van Rooijen N, Sansom OJ, Iredale JP, Lowell S, Roskams T, Forbes SJ. Macrophage-derived Wnt opposes notch signaling to specify hepatic progenitor cell fate in chronic liver disease. *Nat Med* 2012;**18**:572–9
44. Michalopoulos GK, Khan Z. Liver stem cells: experimental findings and implications for human liver disease. *Gastroenterology* 2015;**149**:876–82
45. Tarlow BD, Pelz C, Naugler WE, Wakefield L, Wilson EM, Finegold MJ, Grompe M. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. *Cell Stem Cell* 2014;**15**:605–18
46. Michalopoulos GK, Barua L, Bowen WC. Transdifferentiation of rat hepatocytes into biliary cells after bile duct ligation and toxic biliary injury. *Hepatology* 2005;**41**:535–44
47. Zeng G, Awan F, Otruba W, Muller P, Apte U, Tan X, Gandhi C, Demetris AJ, Monga SP. Wnt'er in liver: expression of Wnt and frizzled genes in mouse. *Hepatology* 2007;**45**:195–204
48. Jiang A, Okabe H, Popovic B, Preziosi ME, Pradhan-Sundd T, Poddar M, Singh S, Bell A, England SG, Nagarajan S, Monga SP. Loss of Wnt secretion by macrophages promotes hepatobiliary injury after administration of 3,5-Diethoxycarbonyl-1, 4-Dihydrocollidine diet. *Am J Pathol* 2019;**189**:590–603
49. Wilson DH, Jarman EJ, Mellin RP, Wilson ML, Waddell SH, Tsokkou P, Younger NT, Raven A, Bhalla SR, Noll ATR, Olde Damink SW, Schaap FG, Chen P, Bates DO, Banales JM, Dean CH, Henderson DJ, Sansom OJ, Kendall TJ, Boulter L. Non-canonical Wnt signalling regulates scarring in biliary disease via the planar cell polarity receptors. *Nat Commun* 2020;**11**:445
50. Irvine KM, Clouston AD, Gadd VL, Miller GC, Wong WY, Melino M, Maradana MR, MacDonald K, Lang RA, Sweet MJ, Blumenthal A, Powell EE. Deletion of Wntless in myeloid cells exacerbates liver fibrosis and the ductular reaction in chronic liver injury. *Fibrogenesis Tissue Repair* 2015;**8**:19
51. Planas-Paz L, Sun T, Pikiolek M, Cochran NR, Bergling S, Orsini V, Yang Z, Sigoillot F, Jetzer J, Syed M, Neri M, Schuierer S, Morelli L, Hoppe PS, Schwarzer W, Cobos CM, Alford JL, Zhang L, Cuttat R,

Waldt A, Carballido-Perrig N, Nigsch F, Kinzel B, Nicholson TB, Yang Y, Mao X, Terracciano LM, Russ C, Reece-Hoyes JS, Gubser Keller C, Sailer AW, Bouwmeester T, Greenbaum LE, Lugus JJ, Cong F, McAllister G, Hoffman GR, Roma G, Tchorz JS. Yap, but not RSPO-LGR4/5, signaling in biliary epithelial cells promotes a ductular reaction in response to liver injury. *Cell Stem Cell* 2019;**25**:39–53 e10

52. Sampaziotis F, Justin AW, Tysoe OC, Sawiak S, Godfrey EM, Upponi SS, Gieseck RL 3rd, de Brito MC, Berntsen NL, Gomez-Vazquez MJ, Ortmann D, Yiangou L, Ross A, Bargehr J, Bertero A, Zonneveld MCF, Pedersen MT, Pawlowski M, Valestrand L, Madrigal P, Georgakopoulos N, Pirmadjid N, Skeldon GM, Casey J, Shu W, Materek PM, Snijders KE, Brown SE, Rimland CA, Simonic I, Davies SE, Jensen KB, Zilbauer M, Gelson WTH, Alexander GJ, Sinha S, Hannan NRF, Wynn TA, Karlsen TH, Melum E, Markaki AE, Saeb-Parsy K, Vallier L. Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. *Nat Med* 2017;**23**:954–63
53. Pepe-Mooney BJ, Dill MT, Alemany A, Ordovas-Montanes J, Matsushita Y, Rao A, Sen A, Miyazaki M, Anakk S, Dawson PA, Ono N, Shalek AK, van Oudenaarden A, Camargo FD. Single-cell analysis of the liver epithelium reveals dynamic heterogeneity and an essential role for Yap in homeostasis and regeneration. *Cell Stem Cell* 2019;**25**:23–38 e8
54. Williams BB, Mundell N, Dunlap J, Jessen J. The planar cell polarity protein VANGL2 coordinates remodeling of the extracellular matrix. *Commun Integr Biol* 2012;**5**:325–8
55. Williams BB, Cantrell VA, Mundell NA, Bennett AC, Quick RE, Jessen JR. VANGL2 regulates membrane trafficking of MMP14 to control cell polarity and migration. *J Cell Sci* 2012;**125**:2141–7
56. Li Z, Takino T, Endo Y, Sato H. Activation of MMP-9 by membrane type-1 MMP/MMP-2 axis stimulates tumor metastasis. *Cancer Sci* 2017;**108**:347–53
57. Poobalasingam T, Yates LL, Walker SA, Pereira M, Gross NY, Ali A, Kolatsi-Joannou M, Jarvelin MR, Pekkanen J, Papakrivopoulou E, Long DA, Griffiths M, Wagner D, Konigshoff M, Hind M, Minelli C, Lloyd CM, Dean CH. Heterozygous Vangl2(looptail) mice reveal novel roles for the planar cell polarity pathway in adult lung homeostasis and repair. *Dis Model Mech* 2017;**10**:409–23
58. Papakrivopoulou E, Vasilopoulou E, Lindenmeyer MT, Pacheco S, Brzoska HL, Price KL, Kolatsi-Joannou M, White KE, Henderson DJ, Dean CH, Cohen CD, Salama AD, Woolf AS, Long DA. Vangl2, a planar cell polarity molecule, is implicated in irreversible and reversible kidney glomerular injury. *J Pathol* 2018;**246**:485–96