**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 75102

**Manuscript Type:** MINIREVIEWS

**Mechanisms of** **ductular reaction in non-alcoholic steatohepatitis**

Chen Y *et al*. Ductular reaction in liver diseases

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**Author contributions:** Chen Y searched the literature and drafted the manuscript; Gao WK checked the manuscript and provided assistance regarding illustrations; Shu YY assisted in designing and polishing the manuscript; Ye J revised the important intellectual content in this manuscript; all authors have read and approved the final manuscript.

**Supported by** the National Natural Science Foundation of China, No. 81770582; and the Graduates’ Innovation Fund, Huazhong University of Science and Technology, No. 2021yjsCXCY106.

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**Received:** January 17, 2022

**Revised:** February 18, 2022

**Accepted:** **April 3, 2022**

**Published online:**

**Abstract**

Non-alcoholic fatty liver disease (NAFLD) is a disease spectrum caused in part by insulin resistance and genetic predisposition. This disease is primarily characterized by excessive lipid accumulation in hepatocytes in the absence of alcohol abuse and other causes of liver damage. Histologically, NAFLD is divided into several periods: simple steatosis, non-alcoholic steatohepatitis (NASH), hepatic fibrosis, cirrhosis, and hepatocellular carcinoma. With the increasing prevalence of obesity and hyperlipidemia, NAFLD has become the main cause of chronic liver disease worldwide. As a result, the pathogenesis of this disease is drawing increasing attention. Ductular reaction (DR) is a reactive bile duct hyperplasia caused by liver injury that involves hepatocytes, cholangiocytes, and hepatic progenitor cells. Recently, DR is shown to play a pivotal role in simple steatosis progression to NASH or liver fibrosis, providing new research and treatment options. This study reviews several DR signaling pathways, including Notch, Hippo/YAP-TAZ, Wnt/β-catenin, Hedgehog, HGF/c-Met, and TWEAK/Fn14, and their role in the occurrence and development of NASH.

**Key Words:** Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Ductular reaction; Mechanisms; Signaling pathways

Chen Y, Gao WK, Shu YY, Ye J. Mechanisms of ductular reaction in non-alcoholic steatohepatitis. *World J Gastroenterol* 2022; In press

**Core Tip:** With the increasing prevalence of obesity and hyperlipidemia, Non-alcoholic fatty liver disease (NAFLD) has become the primary cause of chronic liver disease worldwide. Thus, the pathogenesis of non-alcoholic steatohepatitis (NASH) is drawing increasing attention. Ductular reaction (DR) is a reactive bile duct hyperplasia involving hepatocytes, cholangiocytes, and hepatic progenitor cells, that plays an important role in NAFLD pathogenesis and promotes the occurrence and development of NASH and liver fibrosis. This minireview describes the characteristics of DR and summarizes its pivotal mechanisms. A role for DR during NASH is described that supplements current knowledge about the pathogenesis of this disease and informs potential prevention and treatment strategies.

**INTRODUCTION**

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of > 5% hepatic steatosis in the absence of significant alcohol consumption or other liver disease-induced steatosis. This is one of the most important causes of liver disease worldwide and will likely emerge as the leading cause of end-stage liver disease in the coming decades. The epidemiology and demographic characteristics of NAFLD vary from country to country, correlating with the prevalence of obesity and risk factors for metabolic comorbidities. The global prevalence of NAFLD is currently estimated to be 29.1%, with more than 240 million individuals in China. NAFLD and its complications inflict a heavy financial burden on the global health system, patients, and their families. Thus, it is critical to conduct more research on NAFLD pathogenesis to inform the development of new prevention or treatment strategies[1,2].

As a result of fat accumulation, insulin resistance, and oxidative stress, a subgroup (approximately 20%-30%) of NAFLD, a relatively benign condition, can develop into non-alcoholic steatohepatitis (NASH) within three years. This is defined as the presence of hepatic steatosis with evidence of hepatocyte damage accompanied by inflammation and regeneration, that can progress into liver fibrosis, cirrhosis, and hepatocellular carcinoma[3,4].Chronic hepatocyte death often occurs during NASH, and liver regeneration is a common way to restore normal liver structure characterized by phenotypic fidelity of hepatocytes and/or cholangiocytes. There are two primary mechanisms of liver regeneration. Under physiological conditions, liver parenchymal cells are repaired by the division of adjacent resting hepatocytes. During chronic liver diseases, such as NASH, liver regeneration capacity is impaired, activating the alternate regenerative pathway and resulting in ductular reaction (DR). Under these circumstances, macrophages, hepatic stellate cells (HSCs), and the extracellular matrix (ECM) act together to form the inflammatory micro-environment, releasing inflammatory and pro-fibrotic factors and promoting type I collagen deposition. When this occurs, hepatocytes, cholangiocytes, and hepatic progenitor cells (HPCs) in the Hering duct around the portal vein become activated, resulting in DR and eventually leading to liver fibrosis. HPC expansion is shown to occur in NAFLD patients and is strongly correlated with DR[5,6]. This is a common phenomenon during NASH, affecting the stage of fibrosis and disease progression and prognosis[7,8]. In this study, the signaling pathways and the roles of DR during NASH are explored to better understand the pathogenesis of this disease and provide potential treatment strategies to improve NASH outcomes.

**Definition of DR**

In 1957, Popper *et al*[9] first characterized DR as a ductular reaction involved in both acute and chronic liver diseases and found that it was associated with recovery from liver homeostasis. Cells involved in DR include pre-existing cholangiocytes, HPCs, and hepatocytes, together known as ductular reaction cells (DRCs) (Figure 1)[10]. However, DR does not just manifest as bile duct hyperplasia but also as liver injury and cell microenvironment-dependent liver regeneration. In diseases involving cholangiocyte damage, biliary cell number and function can be compensated by cholangiocyte and/or HPC proliferation and the transdifferentiation of hepatocytes into biliary-like cells. During hepatocyte injury and other related diseases, cholangiocytes can transdifferentiate into hepatocytes *via* HPCs[10,11].DRCs exist in a niche with myofibroblasts and macrophages, and can actively promote liver inflammation and fibrosis. In human diseases such as primary sclerosing cholangitis, alcoholic or non-alcoholic steatohepatitis, and viral hepatitis, the number of DRCs in the liver directly correlates with fibrosis severity. In a mouse model, targeted apoptosis of DRCs alleviates fibrosis, while inhibition of DRC apoptosis aggravates liver fibrosis, suggesting that DR correlates with poor prognosis of liver diseases such as fibrosis and even cirrhosis[12-14]. Thus, research on the mechanism and intervention of DR is of great significance to liver disease treatment. EpCAM and NCAM/SOX9 have been proposed as HPC markers, CK7 and CK19 are used to identify cholangiocytes, and albumin and HNF 4α are regarded as hepatocyte markers (Table 1)[11,15-17]. CK7 immunohistochemistry is positive in HPCs and both initial and late intermediate hepatobiliary cell stages during DR[18]. By calculating the ratio of CK-positive cells in damaged liver tissue using immunostaining, DR can be divided into five grades, and liver injury severity can be predicted. Morphologically DR is divided into three types: (1) regular bile duct structure commonly seen in biliary obstruction; (2) incomplete bile duct structure observed in chronic active hepatitis; and (3) small bile ducts reorganized by bile duct epithelial cells and hepatocytes, seen after submassive liver necrosis[19].This allows the disease type and degree to be defined through the pathological manifestation of DR and informs disease treatment.

**DR and Liver Fibrosis in NASH**

Liver fibrosis is a major complication of almost all types of chronic liver damage including NASH. NAFLD includes three patterns of liver fibrosis according to histological characteristics, portal fibrosis, centrilobular fibrosis, and septal fibrosis[20].In human liver diseases, fibrosis in the portal area is associated with poor prognosis even when the primary site of injury is lobular hepatocytes. Initially, NASH presents as active lobular necroinflammatory and typical centrilobular fibrosis, characterized by pericentral monocyte infiltration, myofibroblast activation, and localized collagen deposition, that can progress to portal fibrosis, septal fibrosis, and eventually lead to liver cirrhosis[7,21]. The DR correlates closely with fibrosis severity during NASH[6].A rat model of liver fibrosis induced by chronic 2-acetylaminofluorene showed that HPC expansion aggravated liver fibrosis by driving myofibroblastic transformation of fibroblasts and/or HSCs in the injured liver[22]. However, whether fibrosis aids the regeneration mediated by HPC or DR exacerbates fibrosis remain unclear[7,23].

***Periportal DR in NASH***

Periportal fibrosis is closely related to NASH progression. Periportal DR is a typical injury response caused by portal inflammatory infiltration that leads to periportal fibrosis[6,24]. Lobular injury activates macrophages to release pro-inflammatory factors such as TNF and IL-1β and promotes formation of the portal inflammatory infiltration microenvironment that is dominated by CD68+ macrophages, CD8+ lymphocytes, collagen I, and laminin, contributing to cell fate. As cell damage increases during NASH, HPC proliferation induces DR around the portal vein. DR activates HSCs and induces HPC differentiation by releasing inflammatory mediators and pro-fibrogenic factors, eventually resulting in periportal fibrosis that resembles biliary fibrosis[25]. Except for hepatic parenchymal and nonparenchymal cells, current studies have confirmed that mixed infiltration of lymphocytes, neutrophils, monocytes, and a small number of eosinophils participate in DR, but the relative number of different cells and their relationship to disease progression is not fully understood[26].

Studies have shown that DR and portal inflammation are closely related during NASH and are independently related to fibrosis. In the portal vein area, inflammatory cells and their mediators affect HPC differentiation and this process affects the balance between liver regeneration and fibrogenesis. In a rat model using a 3,5-methoxycarbonyl-1,4-dihydrocollidine (DDC)-enriched diet, DRCs proliferated at the capillary bile duct and migrated radially from the portal area to the hepatic parenchymal region[27]. This suggests that the portal DR may interact with the centrilobular DR, leading to progressive fibrosis and even septal fibrosis.

***Centrilobular DR in NASH***

DR has primarily been described in the periportal compartment. However, a recent study demonstrated that the DR also occurs in the centrilobular regions, suggesting that hepatocytes mount a metaplastic response to chronic injury and/or chronic ischemia, and promote transformation into a more ductular phenotype rather than a progenitor cell reaction[28]. Studies have shown that centrilobular DR is common in NASH and correlates well with lobular inflammation, hepatocellular ballooning degeneration, Mallory-Denk bodies, and necroinflammation. A cross-sectional analysis supported these findings, showing that centrilobular DR is highly correlated with the stage of fibrosis during adult NASH. Moreover, there was a distinct difference in centrilobular DR frequency between patients with progressive and non-progressive fibrosis, suggesting that centrilobular DR can serve as a histologic marker of fibrosis progression[22].

NASH begins with the accumulation of fatty acids and reactive oxygen species in the central area of the lobules, resulting in hepatocyte mitochondrial injury and activation of nearby macrophages and HSCs. Macrophages can release cytokines like TNF-α that promote insulin resistance and inflammation and result in hepatocyte damage. This can promote the transdifferentiation of hepatocytes into DRCs, the expression of inflammatory mediators and pro-fibrotic cytokines, and the activation of nearby HSCs to secrete type I collagen, eventually resulting in peripheral and subsinus fibrosis in the central area of the lobules[20]. Thus, DR in the central area is associated with liver regeneration and repairment of NASH. Centrilobular DR may be an important driver of fibrosis, in which case the degree of centrilobular DR could serve as a histological marker of fibrosis development[22,29].

**DR mechanisms during NASH**

During chronic liver diseases such as NASH, an alternative pathway for HPC proliferation is activated when hepatic self-renewal becomes impaired. Persistent HPC activation accompanies the recruitment of pro-inflammatory factors and the production of pro-fibrotic factors and results in DR, aiding pathological repair of the liver. HPCs can proliferate and differentiate into hepatocytes or cholangiocytes, and hepatocytes can further transdifferentiate into cholangiocytes (Figure 1). DR promotes liver and biliary fibrosis using similar mechanisms. A series of highly conservative signaling pathways, including Notch, Hippo/YAP-TAZ, Wnt/β-catenin, Hedgehog (Hh), HGF/c-Met, and TWEAK/Fn14, in the DR play an important role in driving HPC activation and/or HSC activation in chronic liver injury (Figure 2).

***Notch signaling during NASH***

The Notch ligands, Jagged (Jagged1, 2) and Delta-like (Delta-like, Dll1, 3, and 4), activate Notch receptors (Notch-1, -4) through cell-cell contact, determining cleavage of the Notch intracellular domain (NICD). NICD binds with CBF1/RBPjK in the nucleus and promotes transcription of several genes including the Hes and Hey-related family of transcription factors and SOX9. During embryonic development, Notch signaling can promote HPC differentiation into cells in the biliary lineage and induce morphogenesis and maturation of the intrahepatic biliary tree. In the DR that occurs during adult liver injury, Notch signaling can determine HPC differentiation into cells in the biliary (Notch activation) or hepatocyte lineage (Notch inhibition). In addition, Notch signaling reprograms hepatocytes into biliary epithelial cells to repair the biliary tract[30,31].

During NASH, inflammatory cells express Notch ligands and promote Notch signaling, increasing FoxO1-induced insulin resistance to regulate glucose production by hepatocytes[32]. The Notch signaling pathway can interact with the mTOR pathway to increase intracellular triglyceride synthesis and lipogenesis by regulating SREBP-1c expression[33]. Thus, it is believed that Notch signaling regulates adipogenesis, steatosis, and insulin resistance, and promotes NAFLD occurrence and progression.

Notch signaling is downregulated following liver cell differentiation in healthy individuals but is upregulated in NASH patients and mice. In Notch-2- and RPB-jK-deficient mice with DDC diet-induced biliary damage, HPC activation is severely impaired, suggesting that Notch signaling plays an essential role in HPC driven biliary repair and biliary tubule formation[34]. One study indicated that continuous Notch signaling during lipid accumulation could induce liver steatosis and promote fibrogenesis, while suppression of Notch signaling could ameliorate liver fibrosis. Notch signaling also correlates strongly with the NAFLD activity score and alanine aminotransferase level, indicating that its activity is related to NAFLD progression to NASH. Notch signaling can directly promote liver fibrosis by activating HSCs through osteopontin and sinusoidal endothelial cells and can indirectly affect liver fibrosis through inflammation and DR[35,36]. In NASH mice induced using a methionine-choline deficient diet, high Notch ligand levels were found in activated HSCs and HPC activation was linked to inflammation and fibrosis. Liver fibrosis is improved by inhibiting Notch signaling, which reduces HPCs/DR expansion and hepatocyte transdifferentiation. Meanwhile, immunohistochemistry showed that about 2% of CK19+ cells were derived from Notch-1 induced Sox9+ hepatocytes reprogramming[37]. Lineage tracing showed that hepatocytes undergo extensive reprogramming to biliary epithelial cells (BECs) in the DR following chronic injury[38]. In summary, liver cell reprogramming into HPCs during the DR is Notch-dependent, and inhibition of Notch signaling improves fibrosis, providing a potential target for NASH treatment.

***Hippo/YAP-TAZ signaling during NASH***

In the canonical mammalian Hippo pathway kinase cascade, the tumor suppressor genes, MST1/2 and LATS1/2, are phosphorylated by the upstream kinase tumor suppressor, Hippo, resulting in YAP and TAZ phosphorylation and inactivation. SAV1 and MOB1A/B can act as adaptor proteins to enhance MST1/2 and LATS1/2 phosphorylation and inactivation, respectively. YAP/TAZ interacts with the DNA-binding transcription factors, TEAD (1-4), to regulate target gene expression, and this can be antagonized by TEAD family corepressors such as VGLL4[39].

Hippo-YAP/TAZ signaling participates in various metabolic processes such as liver glycolysis, gluconeogenesis, fatty acid accumulation, and amino acid metabolism. In mice with an MST1 deletion, fasting and high-fat diet aggravated liver metabolic damage. MST1 overexpression is induced by fasting, which decreases SREBP-1c and improves antioxidant genes expression[40]. A previous study demonstrated that AKT overexpression leads to the development of NAFLD by promoting maturation of the transcription factor, SREBP-1c[41]. Studies have shown a positive feedback loop between the Hippo and AKT signaling pathways to promote the development of NAFLD[42]. Thus, regulation of lipid metabolism by Hippo/YAP-TAZ can induce NAFLD. As a result, researchers have turned to the Hippo pathway as a potential therapeutic mechanism for preventing this disease.

Hippo-YAP signaling is related to DR and regulates regeneration of chronic liver disease by determining cellular fates. One study found that YAP levels were increased in NAFLD patients and mice and were primarily localized in the nucleus of DRCs that expressed progenitor markers, correlating with the degree of fibrosis. This suggests that Hippo/YAP signaling is associated with DR and promotes liver fibrosis rather than effective liver regeneration during NASH[43]. In DDC-injured livers, YAP activation occurs in the process of hepatocyte degeneration, and loss of YAP in hepatocytes results in a significant decrease in DR post-DDC injury. In addition, lineage traced hepatocytes in mice showed that YAP was necessary for hepatocytes to form duct-like structures after DDC injury[44]. In several DR mice models, YAP signaling occurred in CK19+ BEC (facultative liver stem cells) and periportal hepatocytes around the portal vein were activated, confirming that YAP was important for BEC expansion and organoid formation and growth. In mice lacking YAP, Sox9 expression in hepatocytes around the portal vein did not increase significantly, and BEC proliferation was significantly reduced. This indicates that YAP mediates the transdifferentiation of Sox9+ hepatocytes and promotes BEC expansion[45]. Another study showed that Hippo signaling could reprogram hepatocytes into ductal cells with characteristics of hepatic progenitors, supporting the idea that YAP could dedifferentiate hepatocytes and reprogram them into cholangiocytes *via* HPCs[46]. Taken together, these findings indicate that the YAP-driven transcriptional program is critical for liver regeneration and hepatocyte reprogramming towards a progenitor, biliary-like fate following liver injury.

In addition, TAZ levels are elevated in NASH patients and mice. When TAZ is silenced, liver inflammation, fibrosis, and cell death are suppressed in NASH mouse models. In contrast, TAZ expression in hepatocytes induces the Indian Hedgehog (Ihh) pathway in mice, promoting pro-fibrotic gene expression in HSCs and mediating the DR process. Both *in vitro* and *in vivo* data indicate that TAZ promotes NASH progression in hepatocytes largely by inducing the Ihh pathway, while silencing TAZ reverses liver inflammation and fibrosis, with the exception of steatosis[47]. In summary, the Hippo-YAP/TAZ signaling pathway provides a potential therapeutic target to prevent the progression of NAFLD to NASH or improve liver fibrosis when NASH is already occurring.

***Wnt-β catenin signaling in NASH***

Wnt was first discovered in Drosophila, and the canonical Wnt signaling pathway was defined as the Wnt-β-catenin mediated transcription pathway. Wnt ligand binds to Frizzled and co-receptor LRPs and blocks β-catenin degradation, causing the transcription of target genes in a [T-cell](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/t-cell) factor/lymphoid enhancer factor dependent manner[48]. Wnt/β-catenin signaling is important for HPC and hepatoblast proliferation and final differentiation into mature hepatocytes and for maintaining bile duct homeostasis. In the adult liver, Wnt signaling is only activated in hepatocytes around the central vein, and maintains a static state in other areas of the liver. When hepatocytes are impaired, Wnt-β catenin signaling is activated[49,50]. The β-catenin-T cell factor complex regulates cyclin expression and induces the cell-cycle G1/S transition, promoting liver regeneration[51].

β-catenin induces insulin resistance by interacting with FoxO1 during gluconeogenesis, promoting NASH. Inhibiting Wnt signaling reduces body lipid content, inhibits liver gluconeogenesis, and increases hepatic insulin sensitivity in NASH mice. These findings suggest that Wnt/β-catenin signaling is related to NASH pathogenesis[52]. Wnt/β-catenin signaling may induce DRC differentiation into hepatocytes, and inhibiting this pathway may improve liver cirrhosis. Wnt levels are higher in the area around the portal vein following continuous damage, suggesting that Wnt/β-catenin signaling regulates hepatobiliary repair[53]. Studies indicate that CK19+ DRCs are regulated by Notch signaling and finally differentiate into cells in the biliary system. Wnt/β-catenin signaling may change the fate of biliary-derived DRCs and instead induce their differentiation into hepatocytes, aiding liver regeneration. However, some studies suggest that DRCs make little contribution to liver regeneration, and Wnt/β-catenin signaling cannot alter DRC differentiation[54]. The differentiation of quiescent HSCs into active myofibroblasts is similar to the dedifferentiation of adipocytes into preadipocytes (loss of adipogenic properties), requiring Wnt/β-catenin signaling. In turn, inhibition of Wnt/β-catenin signaling can block liver fibrosis[52]. The effect of Wnt/β-catenin signaling on the repair of liver damage through DRC proliferation provides a new potential research target for hepatobiliary diseases. However, a more comprehensive understanding of the mechanism by which DRCs and Wnt/β-catenin signaling contribute to hepatobiliary regeneration and repair remains unclear, and more studies are needed to determine whether Wnt/β-catenin signaling regulates DRC differentiation[27].

***Hh signaling in NASH***

Hh was first discovered in drosophila and shown to be critical for promoting tissue development and maintaining homeostasis. There are three Hh ligands, sonic hedgehog (Shh), Ihh, and desert Hh. In the canonical Hh signaling pathway, Hh ligand binding to the transmembrane receptor relieves its inhibitory effect on smoothed, activating the transcriptional mediator glioma-associated oncogene homologues, Glis (Gli1, Gli2, and Gli3). Gli1 is a signal amplifier of Gli2-mediated transcriptional responses, Gli2 is the primary activator of Hh signaling, and Gli3 is responsible for inhibits Hh signaling. Gli1/2 or Gli3 bind to DNA in the nucleus and regulate the transcription of target genes. The Hh pathway plays an important role in hepatic injury repair and fibrogenesis by regulating HPC and mesenchymal cell proliferation and/or differentiation[55,56].

There are multiple mesenchymal cell types that exist in the liver, of which HSCs play a major role in liver fibrosis. In healthy adult liver, liver resident cells produce few Hh ligands and resting HSCs produce Hh inhibitors. As a result, the Hh signaling pathway is relatively quiescent[57,58]. Hh signaling induces HSCs and Gli1+ peribiliary mesenchymal cell proliferation and acquisition of a myofibroblast phenotype responsible for ECM deposition, thus contributing to fibrosis during chronic liver disease[57,59]. Hh signaling recruits bone marrow-derived monocytes to the liver and promotes their transformation into fibrocytes and induces the epithelial-mesenchymal transition in DRCs in response to chronic liver injury[60]. Hepatocytes are the main source of Shh ligands and in the carbon tetrachloride-induced DR model, Shh and Hippo-YAP1 activity are upregulated and correlate with the regulation of DRC fate for liver regeneration. Shh downstream molecular inhibitor, Gant61, can reduce Shh and Yap signaling thereby inhibiting DR and improving liver injury[61]. Thus, Hh signaling is important for the development of antifibrotic therapies because of its potential to regulate the fibrotic process and interact with the Hippo-YAP signaling pathway.

***HGF/c-Met signaling in NASH***

HGF is a pleiotropic growth factor derived from non-parenchymal cells. HGF combines with the c-Met to activate multiple intracellular signaling pathways that impact cell proliferation, migration, and differentiation. HGF acts as an essential cell mitogen, motogen, and morphogen and plays an important role in mesenchymal-epithelial transformation. C-Met is a receptor tyrosine kinase that activates cell growth and morphogenesis, and HGF/c-Met signaling is strongly associated with epithelial, mesenchymal, and hematological malignancy[62].

HGF expression is upregulated in mice with partial liver excision, promoting liver cell division and maturation. Animal studies have shown that knocking out c-Met during embryonic development hinders liver development and can even result in death. The absence of c-Met and epidermal growth factor receptors arrests liver regeneration and can cause mice to die following partial hepatectomy. These findings highlight the importance of HGF/c-Met signaling in liver regeneration and protection[63,64]. Experiments indicate that HPCs can express c-Met. Inhibiting c-Met phosphorylation inhibits HPC proliferation and the transdifferentiation of hepatocytes into cholangiocytes[65]. HGF also enhances collagenase activity. When the liver is severely damaged, HGF expression is up-regulated, increasing the degradation of collagen fibers and the inhibiting liver fibrosis[66]. HGF induces hepatic progenitor marker gene expression and promotes hepatocyte proliferation and HPC conversion into hepatocytes[67]. These findings suggest that HGF/c-Met signaling can promote regeneration and improve fibrosis during chronic liver injury.

***TWEAK/Fn14 signaling in NASH***

TWEAK is a member of the tumor necrosis factor ligand superfamily. Once bound to its receptor, Fn14 participates in many pivotal cellular activities like tissue inflammation, damage repair, cell survival, and death. TWEAK is produced by many myeloid and immune cells and Fn14 is upregulated by fibroblast-like growth factor and other factors associated with injury and inflammation. While the physiological role of the TWEAK/Fn14 axis is to protect against tissue injury, excessive production of TWEAK or Fn14 can drive and orchestrate inflammation, fibrosis, and tissue remodeling[68]. Previous studies show that TWEAK can promote collagen production and pro-inflammatory cytokine secretion and regulate HSC senescence and migration[69].

TWEAK is widely expressed in adult tissue including activated monocytes, natural killer cells, and macrophages in the liver. TWEAK is a direct mitogen to HPCs, and TWEAK/Fn14 signaling can activate NF-κB and [STAT3](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/stat3) [signaling](https://www.sciencedirect.com/topics/medicine-and-dentistry/signal-transduction) to increase pro-inflammatory [cytokine secretion](https://www.sciencedirect.com/topics/medicine-and-dentistry/cytokine-release) during liver disease[70]. Fn14 expression is relatively low in resting HSCs, but during chronic liver injury, Fn14 is highly expressed in HPCs and activated HSCs[71]. TWEAK promotes HSC migration through activation of the EGFR/Src and PI3K/AKT pathways, which are important for liver fibrogenesis[69]. One study demonstrated that Fn14-deficient mice treated with a choline-deficient, ethionine-supplemented diet had diminished HPC proliferation, inflammation, collagen deposition, and profibrotic cytokine production that resulted in considerably more mild liver fibrosis[72,73].Given its effect on pathological remodeling, HSC migration, and pro-inflammatory cytokine expression, TWEAK/Fn14 signaling may serve as a potential strategy of antifibrotic treatment strategy for NASH by regulating HPC proliferation and fibrogenesis.

***Signaling pathway crosstalk***

Recent studies have supported the view that there is an interactive network between different signaling pathways to regulate DR during NASH. Signaling pathways such as the Hippo/YAP, Wnt/β-catenin, Shh pathway can interact with Notch signaling directly or indirectly to regulate cellular gene expression. Wnt/β-catenin promotes DRCs to differentiate into hepatocytes and inhibits Notch signaling[74]. Both *in vitro* and *in vivo* data indicate that hepatocyte TAZ promotes NASH progression in large part by inducing Ihh signaling, playing an important role in fibrogenesis[47]. Notch signaling is one important downstream YAP target in liver cells[46]. Hippo/YAP-TAZ can upregulate Notch ligands or cooperate with Notch signaling to manipulate target genes in DR[75]. These signaling pathways have distinct roles in DR, including regulating proliferation and transdifferentiation of hepatocytes, cholangiocytes, and HPCs, activating HSCs and fibrogenesis. These pathways can enhance or weaken each other’s effects through complicated signaling networks which will require more in-depth understanding and research.

**CONCLUSION**

In summary, the DR is a response to the bile duct phenotype during liver injury, which aims to restore liver homeostasis and promote regeneration during chronic liver injury by regulating HPC, cholangiocyte, and hepatocyte proliferation and differentiation. The DR plays an important role in NAFLD pathogenesis and promotes the occurrence and development of NASH and liver fibrosis. It is shown that Notch, Hippo/YAP, Wnt/β-catenin, Hh, HGF/c-Met, TWEAK/Fn14, and other intracellular signaling pathways interact with each other to form a crosstalk network, which is related to the DR. In chronic liver disease, these signaling pathways can affect liver inflammation, regeneration, HSC activation, and collagen deposition by regulating corresponding target gene expression. Several molecular inhibitors and modulators are being considered for anti-fibrotic treatment during NASH. While the specific mechanisms for these signaling pathways require more exploration, NASH treatment looks promising in near future.

**ACKNOWLEDGEMENTS**

Figures were created using BioRender ([www.bioreder.com](http://www.bioreder.com)).

**REFERENCES**

1 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

2 **Li J**, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, Fujii H, Wu Y, Kam LY, Ji F, Li X, Chien N, Wei M, Ogawa E, Zhao C, Wu X, Stave CD, Henry L, Barnett S, Takahashi H, Furusyo N, Eguchi Y, Hsu YC, Lee TY, Ren W, Qin C, Jun DW, Toyoda H, Wong VW, Cheung R, Zhu Q, Nguyen MH. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2019; **4**: 389-398 [PMID: 30902670 DOI: 10.1016/S2468-1253(19)30039-1]

3 **Calzadilla Bertot L**, Adams LA. The Natural Course of Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 2016; **17** [PMID: 27213358 DOI: 10.3390/ijms17050774]

4 **Rinella ME**. Nonalcoholic fatty liver disease: a systematic review. *JAMA* 2015; **313**: 2263-2273 [PMID: 26057287 DOI: 10.1001/jama.2015.5370]

5 **Dezső K**, Nagy P, Paku S. Human liver regeneration following massive hepatic necrosis: Two distinct patterns. *J Gastroenterol Hepatol* 2020; **35**: 124-134 [PMID: 31090096 DOI: 10.1111/jgh.14721]

6 **Richardson MM**, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, Weltman MD, Tilg H, Moschen AR, Purdie DM, Demetris AJ, Clouston AD. Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction. *Gastroenterology* 2007; **133**: 80-90 [PMID: 17631134 DOI: 10.1053/j.gastro.2007.05.012]

7 **Clouston AD**, Jonsson JR, Powell EE. Hepatic progenitor cell-mediated regeneration and fibrosis: chicken or egg? *Hepatology* 2009; **49**: 1424-1426 [PMID: 19399908 DOI: 10.1002/hep.22893]

8 **Peverill W**, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *Int J Mol Sci* 2014; **15**: 8591-8638 [PMID: 24830559 DOI: 10.3390/ijms15058591]

9 **Popper H**, Kent G, Stein R. Ductular cell reaction in the liver in hepatic injury. *J Mt Sinai Hosp N Y* 1957; **24**: 551-556 [PMID: 13476145]

10 **Roskams TA**, Theise ND, Balabaud C, Bhagat G, Bhathal PS, Bioulac-Sage P, Brunt EM, Crawford JM, Crosby HA, Desmet V, Finegold MJ, Geller SA, Gouw AS, Hytiroglou P, Knisely AS, Kojiro M, Lefkowitch JH, Nakanuma Y, Olynyk JK, Park YN, Portmann B, Saxena R, Scheuer PJ, Strain AJ, Thung SN, Wanless IR, West AB. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology* 2004; **39**: 1739-1745 [PMID: 15185318 DOI: 10.1002/hep.20130]

11 **Sato K**, Marzioni M, Meng F, Francis H, Glaser S, Alpini G. Ductular Reaction in Liver Diseases: Pathological Mechanisms and Translational Significances. *Hepatology* 2019; **69**: 420-430 [PMID: 30070383 DOI: 10.1002/hep.30150]

12 **Krishnan A**, Katsumi T, Guicciardi ME, Azad AI, Ozturk NB, Trussoni CE, Gores GJ. Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptor Deficiency Promotes the Ductular Reaction, Macrophage Accumulation, and Hepatic Fibrosis in the Abcb4-/- Mouse. *Am J Pathol* 2020; **190**: 1284-1297 [PMID: 32240619 DOI: 10.1016/j.ajpath.2020.02.013]

13 **Bird TG**, Lu WY, Boulter L, Gordon-Keylock S, Ridgway RA, Williams MJ, Taube J, Thomas JA, Wojtacha D, Gambardella A, Sansom OJ, Iredale JP, Forbes SJ. Bone marrow injection stimulates hepatic ductular reactions in the absence of injury via macrophage-mediated TWEAK signaling. *Proc Natl Acad Sci U S A* 2013; **110**: 6542-6547 [PMID: 23576749 DOI: 10.1073/pnas.1302168110]

14 **Azad AI**, Krishnan A, Troop L, Li Y, Katsumi T, Pavelko K, Kostallari E, Guicciardi ME, Gores GJ. Targeted Apoptosis of Ductular Reactive Cells Reduces Hepatic Fibrosis in a Mouse Model of Cholestasis. *Hepatology* 2020; **72**: 1013-1028 [PMID: 32128842 DOI: 10.1002/hep.31211]

15 **Segal JM**, Kent D, Wesche DJ, Ng SS, Serra M, Oulès B, Kar G, Emerton G, Blackford SJI, Darmanis S, Miquel R, Luong TV, Yamamoto R, Bonham A, Jassem W, Heaton N, Vigilante A, King A, Sancho R, Teichmann S, Quake SR, Nakauchi H, Rashid ST. Single cell analysis of human foetal liver captures the transcriptional profile of hepatobiliary hybrid progenitors. *Nat Commun* 2019; **10**: 3350 [PMID: 31350390 DOI: 10.1038/s41467-019-11266-x]

16 **Schmelzer E**, Pietrosi G, Gridelli B, Gerlach J. Characterization of CD326-positive human hepatic stem cells. *Clin Exp Hepatol* 2021; **7**: 101-110 [PMID: 34027122 DOI: 10.5114/ceh.2021.104459]

17 **Abou Monsef Y**, Kutsal O. Immunohistochemical evaluation of hepatic progenitor cells in different types of feline liver diseases. *J Vet Med Sci* 2021; **83**: 613-621 [PMID: 33583913 DOI: 10.1292/jvms.20-0435]

18 **Misra S**, Majumdar K, Sakhuja P, Jain P, Singh L, Kumar P, Dubey AP. Differentiating Biliary Atresia From Idiopathic Neonatal Hepatitis: A Novel Keratin 7 Based Mathematical Approach on Liver Biopsies. *Pediatr Dev Pathol* 2021; **24**: 103-115 [PMID: 33439108 DOI: 10.1177/1093526620983730]

19 **Zheng** **YJ**. Bile duct reaction related to clinical hepatobiliary diseases and its significance. *Zhonghua Ganzang Bing Zazhi* 2018; **26**: 637-640

20 **Skoien R**, Richardson MM, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, O'Brien PE, Tilg H, Moschen AR, Baumann U, Brown RM, Couper RT, Manton ND, Ee LC, Weltman M, Clouston AD. Heterogeneity of fibrosis patterns in non-alcoholic fatty liver disease supports the presence of multiple fibrogenic pathways. *Liver Int* 2013; **33**: 624-632 [PMID: 23356584 DOI: 10.1111/liv.12100]

21 **Melino M**, Gadd VL, Alexander KA, Beattie L, Lineburg KE, Martinez M, Teal B, Le Texier L, Irvine KM, Miller GC, Boyle GM, Hill GR, Clouston AD, Powell EE, MacDonald KP. Spatiotemporal Characterization of the Cellular and Molecular Contributors to Liver Fibrosis in a Murine Hepatotoxic-Injury Model. *Am J Pathol* 2016; **186**: 524-538 [PMID: 26762581 DOI: 10.1016/j.ajpath.2015.10.029]

22 **Zhao L**, Westerhoff M, Pai RK, Choi WT, Gao ZH, Hart J. Centrilobular ductular reaction correlates with fibrosis stage and fibrosis progression in non-alcoholic steatohepatitis. *Mod Pathol* 2018; **31**: 150-159 [PMID: 28862262 DOI: 10.1038/modpathol.2017.115]

23 **Williams MJ**, Clouston AD, Forbes SJ. Links between hepatic fibrosis, ductular reaction, and progenitor cell expansion. *Gastroenterology* 2014; **146**: 349-356 [PMID: 24315991 DOI: 10.1053/j.gastro.2013.11.034]

24 **Gouw AS**, Clouston AD, Theise ND. Ductular reactions in human liver: diversity at the interface. *Hepatology* 2011; **54**: 1853-1863 [PMID: 21983984 DOI: 10.1002/hep.24613]

25 **Gadd VL**, Skoien R, Powell EE, Fagan KJ, Winterford C, Horsfall L, Irvine K, Clouston AD. The portal inflammatory infiltrate and ductular reaction in human nonalcoholic fatty liver disease. *Hepatology* 2014; **59**: 1393-1405 [PMID: 24254368 DOI: 10.1002/hep.26937]

26 **Brunt EM**, Kleiner DE, Wilson LA, Unalp A, Behling CE, Lavine JE, Neuschwander-Tetri BA; NASH Clinical Research NetworkA list of members of the Nonalcoholic Steatohepatitis Clinical Research Network can be found in the Appendix. Portal chronic inflammation in nonalcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD-Clinicopathologic correlations from the nonalcoholic steatohepatitis clinical research network. *Hepatology* 2009; **49**: 809-820 [PMID: 19142989 DOI: 10.1002/hep.22724]

27 **Wang N**, Kong R, Han W, Lu J. Wnt/β-catenin signalling controls bile duct regeneration by regulating differentiation of ductular reaction cells. *J Cell Mol Med* 2020; **24**: 14050-14058 [PMID: 33124779 DOI: 10.1111/jcmm.16017]

28 **Gill RM**, Belt P, Wilson L, Bass NM, Ferrell LD. Centrizonal arteries and microvessels in nonalcoholic steatohepatitis. *Am J Surg Pathol* 2011; **35**: 1400-1404 [PMID: 21836480 DOI: 10.1097/PAS.0b013e3182254283]

29 **Nagahama Y**, Sone M, Chen X, Okada Y, Yamamoto M, Xin B, Matsuo Y, Komatsu M, Suzuki A, Enomoto K, Nishikawa Y. Contributions of hepatocytes and bile ductular cells in ductular reactions and remodeling of the biliary system after chronic liver injury. *Am J Pathol* 2014; **184**: 3001-3012 [PMID: 25193593 DOI: 10.1016/j.ajpath.2014.07.005]

30 **Bray SJ**. Notch signalling in context. *Nat Rev Mol Cell Biol* 2016; **17**: 722-735 [PMID: 27507209 DOI: 10.1038/nrm.2016.94]

31 **Geisler F**, Strazzabosco M. Emerging roles of Notch signaling in liver disease. *Hepatology* 2015; **61**: 382-392 [PMID: 24930574 DOI: 10.1002/hep.27268]

32 **Pajvani UB**, Shawber CJ, Samuel VT, Birkenfeld AL, Shulman GI, Kitajewski J, Accili D. Inhibition of Notch signaling ameliorates insulin resistance in a FoxO1-dependent manner. *Nat Med* 2011; **17**: 961-967 [PMID: 21804540 DOI: 10.1038/nm.2378]

33 **Bi P**, Kuang S. Notch signaling as a novel regulator of metabolism. *Trends Endocrinol Metab* 2015; **26**: 248-255 [PMID: 25805408 DOI: 10.1016/j.tem.2015.02.006]

34 **Fiorotto R**, Raizner A, Morell CM, Torsello B, Scirpo R, Fabris L, Spirli C, Strazzabosco M. Notch signaling regulates tubular morphogenesis during repair from biliary damage in mice. *J Hepatol* 2013; **59**: 124-130 [PMID: 23500150 DOI: 10.1016/j.jhep.2013.02.025]

35 **Valenti L**, Mendoza RM, Rametta R, Maggioni M, Kitajewski C, Shawber CJ, Pajvani UB. Hepatic notch signaling correlates with insulin resistance and nonalcoholic fatty liver disease. *Diabetes* 2013; **62**: 4052-4062 [PMID: 23990360 DOI: 10.2337/db13-0769]

36 **Zhu C**, Kim K, Wang X, Bartolome A, Salomao M, Dongiovanni P, Meroni M, Graham MJ, Yates KP, Diehl AM, Schwabe RF, Tabas I, Valenti L, Lavine JE, Pajvani UB. Hepatocyte Notch activation induces liver fibrosis in nonalcoholic steatohepatitis. *Sci Transl Med* 2018; **10** [PMID: 30463916 DOI: 10.1126/scitranslmed.aat0344]

37 **Morell CM**, Fiorotto R, Meroni M, Raizner A, Torsello B, Cadamuro M, Spagnuolo G, Kaffe E, Sutti S, Albano E, Strazzabosco M. Notch signaling and progenitor/ductular reaction in steatohepatitis. *PLoS One* 2017; **12**: e0187384 [PMID: 29140985 DOI: 10.1371/journal.pone.0187384]

38 **Yanger K**, Zong Y, Maggs LR, Shapira SN, Maddipati R, Aiello NM, Thung SN, Wells RG, Greenbaum LE, Stanger BZ. Robust cellular reprogramming occurs spontaneously during liver regeneration. *Genes Dev* 2013; **27**: 719-724 [PMID: 23520387 DOI: 10.1101/gad.207803.112]

39 **Driskill JH**, Pan D. The Hippo Pathway in Liver Homeostasis and Pathophysiology. *Annu Rev Pathol* 2021; **16**: 299-322 [PMID: 33234023 DOI: 10.1146/annurev-pathol-030420-105050]

40 **Geng C**, Zhang Y, Gao Y, Tao W, Zhang H, Liu X, Fang F, Chang Y. Mst1 regulates hepatic lipid metabolism by inhibiting Sirt1 ubiquitination in mice. *Biochem Biophys Res Commun* 2016; **471**: 444-449 [PMID: 26903296 DOI: 10.1016/j.bbrc.2016.02.059]

41 **Krycer JR**, Sharpe LJ, Luu W, Brown AJ. The Akt-SREBP nexus: cell signaling meets lipid metabolism. *Trends Endocrinol Metab* 2010; **21**: 268-276 [PMID: 20117946 DOI: 10.1016/j.tem.2010.01.001]

42 **Jeong SH**, Kim HB, Kim MC, Lee JM, Lee JH, Kim JH, Kim JW, Park WY, Kim SY, Kim JB, Kim H, Kim JM, Choi HS, Lim DS. Hippo-mediated suppression of IRS2/AKT signaling prevents hepatic steatosis and liver cancer. *J Clin Invest* 2018; **128**: 1010-1025 [PMID: 29400692 DOI: 10.1172/JCI95802]

43 **Machado MV**, Michelotti GA, Pereira TA, Xie G, Premont R, Cortez-Pinto H, Diehl AM. Accumulation of duct cells with activated YAP parallels fibrosis progression in non-alcoholic fatty liver disease. *J Hepatol* 2015; **63**: 962-970 [PMID: 26070409 DOI: 10.1016/j.jhep.2015.05.031]

44 **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 [PMID: 31080134 DOI: 10.1016/j.stem.2019.04.004]

45 **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 [PMID: 31080135 DOI: 10.1016/j.stem.2019.04.005]

46 **Yimlamai D**, Christodoulou C, Galli GG, Yanger K, Pepe-Mooney B, Gurung B, Shrestha K, Cahan P, Stanger BZ, Camargo FD. Hippo pathway activity influences liver cell fate. *Cell* 2014; **157**: 1324-1338 [PMID: 24906150 DOI: 10.1016/j.cell.2014.03.060]

47 **Wang X**, Zheng Z, Caviglia JM, Corey KE, Herfel TM, Cai B, Masia R, Chung RT, Lefkowitch JH, Schwabe RF, Tabas I. Hepatocyte TAZ/WWTR1 Promotes Inflammation and Fibrosis in Nonalcoholic Steatohepatitis. *Cell Metab* 2016; **24**: 848-862 [PMID: 28068223 DOI: 10.1016/j.cmet.2016.09.016]

48 **Tortelote GG**, Reis RR, de Almeida Mendes F, Abreu JG. Complexity of the Wnt/β‑catenin pathway: Searching for an activation model. *Cell Signal* 2017; **40**: 30-43 [PMID: 28844868 DOI: 10.1016/j.cellsig.2017.08.008]

49 **Benhamouche S**, Decaens T, Godard C, Chambrey R, Rickman DS, Moinard C, Vasseur-Cognet M, Kuo CJ, Kahn A, Perret C, Colnot S. Apc tumor suppressor gene is the "zonation-keeper" of mouse liver. *Dev Cell* 2006; **10**: 759-770 [PMID: 16740478 DOI: 10.1016/j.devcel.2006.03.015]

50 **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**: 1579-1591 [PMID: 17983805 DOI: 10.1053/j.gastro.2007.08.036]

51 **Monga SP**. Role and regulation of β-catenin signaling during physiological liver growth. *Gene Expr* 2014; **16**: 51-62 [PMID: 24801166 DOI: 10.3727/105221614X13919976902138]

52 **Monga SP**. β-Catenin Signaling and Roles in Liver Homeostasis, Injury, and Tumorigenesis. *Gastroenterology* 2015; **148**: 1294-1310 [PMID: 25747274 DOI: 10.1053/j.gastro.2015.02.056]

53 **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**: 1652-1666 [PMID: 27533619 DOI: 10.1002/hep.28774]

54 **Jörs S**, Jeliazkova P, Ringelhan M, Thalhammer J, Dürl S, Ferrer J, Sander M, Heikenwalder M, Schmid RM, Siveke JT, Geisler F. Lineage fate of ductular reactions in liver injury and carcinogenesis. *J Clin Invest* 2015; **125**: 2445-2457 [PMID: 25915586 DOI: 10.1172/JCI78585]

55 **Kopinke D**, Norris AM, Mukhopadhyay S. Developmental and regenerative paradigms of cilia regulated hedgehog signaling. *Semin Cell Dev Biol* 2021; **110**: 89-103 [PMID: 32540122 DOI: 10.1016/j.semcdb.2020.05.029]

56 **Briscoe J**, Thérond PP. The mechanisms of Hedgehog signalling and its roles in development and disease. *Nat Rev Mol Cell Biol* 2013; **14**: 416-429 [PMID: 23719536 DOI: 10.1038/nrm3598]

57 **Omenetti A**, Choi S, Michelotti G, Diehl AM. Hedgehog signaling in the liver. *J Hepatol* 2011; **54**: 366-373 [PMID: 21093090 DOI: 10.1016/j.jhep.2010.10.003]

58 **Sicklick JK**, Li YX, Melhem A, Schmelzer E, Zdanowicz M, Huang J, Caballero M, Fair JH, Ludlow JW, McClelland RE, Reid LM, Diehl AM. Hedgehog signaling maintains resident hepatic progenitors throughout life. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G859-G870 [PMID: 16322088 DOI: 10.1152/ajpgi.00456.2005]

59 **Gupta V**, Gupta I, Park J, Bram Y, Schwartz RE. Hedgehog Signaling Demarcates a Niche of Fibrogenic Peribiliary Mesenchymal Cells. *Gastroenterology* 2020; **159**: 624-638.e9 [PMID: 32289375 DOI: 10.1053/j.gastro.2020.03.075]

60 **Shen X**, Peng Y, Li H. The Injury-Related Activation of Hedgehog Signaling Pathway Modulates the Repair-Associated Inflammation in Liver Fibrosis. *Front Immunol* 2017; **8**: 1450 [PMID: 29163520 DOI: 10.3389/fimmu.2017.01450]

61 **Jin L**, Huang H, Ni J, Shen J, Liu Z, Li L, Fu S, Yan J, Hu B. Shh-Yap signaling controls hepatic ductular reactions in CCl4 -induced liver injury. *Environ Toxicol* 2021; **36**: 194-203 [PMID: 32996673 DOI: 10.1002/tox.23025]

62 **Ma Y**, Yu X, Li YX, Wang YL. HGF/c-Met signaling regulates early differentiation of placental trophoblast cells. *J Reprod Dev* 2021; **67**: 89-97 [PMID: 33455972 DOI: 10.1262/jrd.2020-107]

63 **Jeon HM**, Lee J. MET: roles in epithelial-mesenchymal transition and cancer stemness. *Ann Transl Med* 2017; **5**: 5 [PMID: 28164090 DOI: 10.21037/atm.2016.12.67]

64 **Paranjpe S**, Bowen WC, Mars WM, Orr A, Haynes MM, DeFrances MC, Liu S, Tseng GC, Tsagianni A, Michalopoulos GK. Combined systemic elimination of MET and epidermal growth factor receptor signaling completely abolishes liver regeneration and leads to liver decompensation. *Hepatology* 2016; **64**: 1711-1724 [PMID: 27397846 DOI: 10.1002/hep.28721]

65 **Zang JF,** Yuan Y, Gao JY, Qian HX. The role of HGF/c-Met related signal pathways in the proliferation of rat liver progenitor cells. *Zhongguo Laonian Xue Zazhi* 2014; **34**: 2800-2803

66 **Wu ZH,** Meng X, Hu JW, Ding YF, Peng YR. Research progress on the correlation between TLR4-MyD88-NF-kB signaling pathway and hepatitis-hepatic fibrosis-hepatocellular carcinoma axis. *J Int Pharm Res* 2017; **44**: 396-401 [DOI: 10.13220/j.cnki.jipr.2017.05.003]

67 **Huang Y**, Miyamoto D, Li PL, Sakai Y, Hara T, Adachi T, Soyama A, Hidaka M, Kanetaka K, Gu WL, Eguchi S. Chemical conversion of aged hepatocytes into bipotent liver progenitor cells. *Hepatol Res* 2021; **51**: 323-335 [PMID: 33378128 DOI: 10.1111/hepr.13609]

68 **Croft M**, Siegel RM. Beyond TNF: TNF superfamily cytokines as targets for the treatment of rheumatic diseases. *Nat Rev Rheumatol* 2017; **13**: 217-233 [PMID: 28275260 DOI: 10.1038/nrrheum.2017.22]

69 **Zhang F**, Xu M, Yin X, Guo H, Zhang B, Wang Y, Xiao J, Zou X, Zhang M, Zhuge Y. TWEAK promotes hepatic stellate cell migration through activating EGFR/Src and PI3K/AKT pathways. *Cell Biol Int* 2019 [PMID: 31498529 DOI: 10.1002/cbin.11230]

70 **Wang A**, Zhang F, Xu H, Xu M, Cao Y, Wang C, Xu Y, Su M, Zhang M, Zhuge Y. TWEAK/Fn14 promotes pro-inflammatory cytokine secretion in hepatic stellate cells via NF-κB/STAT3 pathways. *Mol Immunol* 2017; **87**: 67-75 [PMID: 28411440 DOI: 10.1016/j.molimm.2017.04.003]

71 **Zhang Y**, Zeng W, Xia Y. TWEAK/Fn14 axis is an important player in fibrosis. *J Cell Physiol* 2021; **236**: 3304-3316 [PMID: 33000480 DOI: 10.1002/jcp.30089]

72 **Tirnitz-Parker JE**, Viebahn CS, Jakubowski A, Klopcic BR, Olynyk JK, Yeoh GC, Knight B. Tumor necrosis factor-like weak inducer of apoptosis is a mitogen for liver progenitor cells. *Hepatology* 2010; **52**: 291-302 [PMID: 20578156 DOI: 10.1002/hep.23663]

73 **Tirnitz-Parker JE**, Olynyk JK, Ramm GA. Role of TWEAK in coregulating liver progenitor cell and fibrogenic responses. *Hepatology* 2014; **59**: 1198-1201 [PMID: 24038142 DOI: 10.1002/hep.26701]

74 **Zhu C**, Tabas I, Schwabe RF, Pajvani UB. Maladaptive regeneration - the reawakening of developmental pathways in NASH and fibrosis. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 131-142 [PMID: 33051603 DOI: 10.1038/s41575-020-00365-6]

75 **Schwabe RF**, Tabas I, Pajvani UB. Mechanisms of Fibrosis Development in Nonalcoholic Steatohepatitis. *Gastroenterology* 2020; **158**: 1913-1928 [PMID: 32044315 DOI: 10.1053/j.gastro.2019.11.311]

**Footnotes**

**Conflict-of-interest statement:** The authors declare no conflict of interest for this article.

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**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review started:** January 17, 2022

**First decision:** February 7, 2022

**Article in press:**

**Specialty type:** Medicine, research and experimental

**Country/Territory of origin:** China

**Peer-review report’s scientific quality classification**

Grade A (Excellent): A

Grade B (Very good): 0

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Kotlyarov S, Russia; Moldovan C, Romania **S-Editor:** Zhang H **L-Editor:** A **P-Editor:** Zhang H

**Figure Legends**

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**Figure 1 The different compartments of the ductular reaction, including hepatocyte self-proliferation and transdifferentiation, hepatic progenitor cell differentiation, and cholangiocyte proliferation and transdifferention.** HPC: Hepatic progenitor cell.



**Figure 2 A series of highly conserved signaling pathways in the ductular reaction which promotes the occurrence of non-alcoholic fatty liver disease and aggravates the prognosis of non-alcoholic steatohepatitis (Created with BioRender.com).** A: The Notch signaling pathway regulates expression of genes, such as the Hes and Hey-related family, to determine cell differentiation and function, maintain liver homeostasis, repair liver damage, and regulate liver metabolism, inflammation, and cancer; B: The Hippo/YAP-TAZ signaling pathway can regulate liver size, metabolism, cell proliferation, cell migration, the epithelial-mesenchymal transition, and formation of the extracellular matrix and cytoskeleton formation, *etc*; C: The Wnt/β-catenin signaling pathway affects liver development and physiological functions of all liver disease stages, from initial injury and inflammation to fibrosis, cirrhosis and tumor occurrence; D: The hedgehog signaling pathway affects cell proliferation, migration, and differentiation; E: The HGF/c-Met signaling pathway activates multiple intracellular signaling pathways and affect cell proliferation, migration, and differentiation; F: The TWEAK/Fn14 signaling pathway regulates tissue inflammation and damage repair in addition to cell survival and death. HPC: Hepatic progenitor cell; NASH: Non-alcoholic steatohepatitis.

**Table 1 Identifying markers of cholangiocytes, hepatic progenitor cells, and hepatocytes**

|  |  |  |
| --- | --- | --- |
| **Cell types** | **Markers** | **Ref.** |
| Cholangiocytes | CK7/CK19/EpCAM/SOX9/TFF/*etc*. | [15-17] |
| Hepatic progenitor cells | EpCAM/NCAM/SOX9/MCAM/Foxl1/Lgr5/A6/OV6/NGA2/CXCR4/*etc*. |
| TROP2/CD24/CD133/CLDN3/FGFR2/CK7/CK19/SPP1/*etc*. (biliary progenitors) |
| ALB/AFP/DLK1/APOE/TF/HNF4α/*etc*. (hepatic progenitors) |
| Hepatocytes | ALB/HNF4α/RBP4/PCNA/Ki67/Hep Par-1/ASGPR1/*etc*. |