Cholestatic Liver Disease

Subjects: Cell Biology

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Cholestatic liver diseases including primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are associated with active hepatic fibrogenesis, which can ultimately lead to the development of cirrhosis.

cholangiocytes hepatic stellate cells (HSCs) periductular fibroblasts

1. Introduction

Cholestasis is a chronic liver disease characterised by bile flow obstruction in the liver, bile acid (BA) accumulation, and increased BA concentration in the systemic circulation. Thus, during cholestasis impaired bile formation and processing with insufficient bile reaching the duodenum, leads to the accumulation of intrahepatic and systemic BAs and other potentially toxic cholephilic bacteria. The aetiology of cholestasis includes disorders of bile secretion by hepatocytes and/or biliary epithelial cells (BECs), mechanical processes (e.g., stones, tumours) destroying/blocking smaller and/or larger intrahepatic bile ducts, or immune-mediated fibrotic cholangitis, such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) [1][2]. Therefore, BA metabolism plays an important role since the obstruction of bile flow leads to cholestatic injuries, such as in PBC and PSC [3].

Therapies for these diseases are, however, limited. Although ursodeoxycholic acid (UDCA) treatment can significantly improve the prognosis of PBC patients and prolong transplant-free survival, the treatment options for those who do not respond to UDCA remain scarce. Other drugs associated with novel therapies in cholestatic diseases (PBC/PSC) are still in clinical trials, including obeticholic acid (OCA) [4], all-trans retinoic acid (ATRA) [5], Bezafibrate [6], Fenofibrate [7], etc. [8][9]. In addition, there are currently no available drugs to treat PSC [10]. Many of these disorders become chronic, therefore leading eventually to biliary cirrhosis and the need for liver transplantation [11]. Cholestatic liver disease can also cause liver failure and increase the risk of hepatocellular carcinoma (HCC) or cholangiocarcinoma (CCA) [12][13].

2. Primary Biliary Cholangitis (PBC)

PBC, formerly known as primary biliary cirrhosis, is acquired chronic cholestasis related to the autoimmune destruction of small bile ducts, causing portal vein infiltration and fibrosis. PBC is a chronic progressive disease that leads to end-stage liver disease and its related complications [14][15][16]. It can progress to biliary cirrhosis, portal hypertension, liver failure, and is associated with esophagogastric variceal bleeding, ascites, and hepatic encephalopathy [17]. PBC is a destructive lymphocytic cholangitis and specific antimitochondrial antibodies (AMAs) target specific mitochondrial autoantigens [18]. It is characterised by the progressive damage and destruction of

biliary epithelial cells (BECs; also called cholangiocytes) and increased portal vein inflammation and fibrosis [19][20], with chronic histological evidence, nonsuppurative, granulomatous, and lymphocytic cholangitis [17]. Simultaneously, in association with PBC, there are also symptoms that markedly affect the quality of life, including cholestatic pruritus, Sjogren's syndrome, abdominal discomfort, and fatigue [21][22].

Approximately 95% of PBC patients are middle-aged women [23]. Interestingly, this disease rarely affects children [24]. The reports vary worldwide from 1970 to 2014, the annual incidence ranges from 0.3 to 5.8 per 100,000, and the prevalence rates range from 1.9 to 40.2 per 100,000 individuals, respectively, due to increased incidence and improved survival [25][26][27]. From 2004 to 2014, in the United States, the prevalence of PBC increased significantly from 21.7 to 39.2 per 100,000, of which women rose from 33.5 to 57.8 per 100,000 (an increase of 72%), while the incidence rate in men increased from 7.2 to 15.4 per 100,000 (an increase of 114%) [28].

Risk factors for PBC include genetic factors such as the human leukocyte antigen (HLA) and non-HLA allelic variants) [29][30], as well as environmental stimuli. In addition to the regional differences in disease prevalence and family risk, the relationship between epidemiology and bacterial infection, xenobiotics, and smoking history also emphasises the importance of environmental triggers in the pathogenesis of PBC [31][32][33][34][35][36][37]. Furthermore, PBC development has also been linked to microRNA [38] and epigenetic regulation [18]. Moreover, the gut–liver axis is also involved in PBC development. Intestinal dysbacteriosis can affect the bile acid pool and regulate bile acid-activated receptors, which disturbs bile acid metabolism [39][40]. Simultaneously, several lines of evidence suggested that dysbiosis of gut microbiota can destroy the immune homeostasis, thus promoting PBC [41][42].

Population-based historical data from the UK show that about 25% of untreated "classic PBC" patients develop chronic liver failure during this period [43]. An early prospective study found that more than 50% of patients with stage I-III PBC developed histologically confirmed cirrhosis within four years [44]. As cirrhotic individuals, PBC patients may develop complications due to the chronic nature of the disease. The presence of cirrhosis, regardless of its aetiology, is a major risk factor for hepatocellular carcinoma (HCC) or cholangiocarcinoma (CCA) [13][45].

3. Primary Sclerosing Cholangitis (PSC)

PSC is associated with liver damage, characterised by intrahepatic or extrahepatic bile duct injury, and fibrosis of the bile ducts inside and outside the liver, resulting in strictures of the bile ducts and obstruction of bile flow. Clinical manifestations reflect the potential sequence of bile duct injury and fibrosis leading to stricture, cholestasis, and biliary cirrhosis with progressive liver dysfunction [46]. PSC is a male-dominant disease when it is associated with inflammatory bowel disease (IBD), (65–70%), with a male-female ratio of approximately 2:1 [23][47][48][49]. Epidemiological studies show that the prevalence of PSC is about 1/10,000 cases globally, while the incidence rate in northern Europe and the United States is 0.4/100,000 to 2.0/100,000 per year [50][51]. Simultaneously, the survival rate of PSC is increasing [47][52][53][54], which may be partly attributed to early diagnosis due to the application of magnetic resonance cholangiography (MRC). The clinical characteristics of newly diagnosed patients

remain stable over time, while no new diagnostic methods were introduced during this period such as before fibrosis occurs [47].

PSC is a typical complex disease with genetic and environmental risk factors. This important genome-wide association has shown that PSC risk is associated with certain phenotypes of human leukocyte antigens (HLA), particularly HLA-DR6, HLA-DR3, and HLA-B8, suggesting the presence of autoimmune disorders in patients with PSC [55]. The risk of PSC is also associated with, at least, 23 regions of the genome [56]. At present, no clear causal environmental factor has been identified, however, the geographical distribution of the disease in northern Europe provides some clues to consider the source of environmental risk factors [57]. Indeed, differences in lifestyle, diet, and living conditions are highly regional [46]. Clinically, inflammatory bowel disease (IBD) is the strongest condition associated with PSC—approximately 70% of patients with PSC having also IBD [58][59][60].

Bacterial cholangitis, osteoporosis, liver cirrhosis, and IBD can be caused by the progression of PSC [61][62][63][64][65][66][67]. It can lead to colorectal neoplasia, pancreatic cancer, CCA, and gallbladder carcinoma [58][65][66][68][69][70]. Large population-based studies indicate that the risk of death in patients with PSC is increased fourfold, in comparison with the general population [47]. The most common causes of death associated with PSC are CCA (32%), liver failure (15%), transplant-related complications (9%), and colorectal cancer (8%), demonstrating that the increased risk of malignancy for PSC has a significant impact on life expectancy [71].

4. Signalling Pathways Involved in Pathogenesis

After cholangiocyte injury, infiltration of immune cells occurs, activating Notch, hedgehog (Hh), Wnt, and other signalling pathways—inducing the increase in fibroblasts due to HSCs activation, which further promote BECs proliferation. Subsequent pathological sequelae including biliary stricture formation, bile retention, bile-related toxic stress, inflammatory cells, or formation of immune cells around the bile duct further aggravate the disease.

4.1. Notch Signalling Pathway

In the past few years, the mechanisms and influence of Notch signalling in liver fibrosis have been developed. Rat HSCs express the Notch receptor in vitro and begin to express JAG1 after activation and differentiation into myofibroblast-like cells [72]. The expression of Notch 2/3, Hey 1/2 increases significantly in the process leading from quiescent HSCs into activated myofibroblasts [73]. High activation of the Notch signal was also observed in hepatic progenitor cells isolated from tissues with PBC [74]. In addition, the number of Notch 1/3/4-positive cells increased significantly in the fibrotic area of Chemokine (C-C motif) ligands 4 (CCl_4)-injured rats [75]. In the CCl_4-induced rat liver fibrosis model, Notch signalling was also hyperactivated [76]. Moreover, inhibition of Notch in CCl_4-induced liver injury significantly damaged HSC activation and triggered the development of fibrosis, and inhibition of this pathway in the liver can prevent or ameliorate fibrosis [77].

During liver fibrosis development, Notch interacts with other signalling pathways, such as TGF-β, Hippo, and Hh, whilst crosstalk between TGF-β activation and Notch occurs in liver fibrosis [78][79] (Figure 1). TGF-β in the liver

may partly promote fibrosis by stimulating Notch activity in HSCs. KCs together with bone marrow-derived macrophages are thought to be the main source of TGF-β1 thus promoting the development of liver fibrosis [80][81]. Recent studies have shown that TGF-β2-induced expression of fibrosis genes in cholangiocytes and HSCs is related to the specific regulation of the Notch3 signalling pathway [82]. SOX9, a transdifferentiated biomarker of BECs specifically expressed in cholangiocytes, is also a downstream target of Notch signalling. After BDL in rats, Notch receptor activation, combined with overexpression of SOX9, enhanced BEC proliferation and induced hyper-hepatic fibrosis [83]. Recently, another study from Athwal et al. [84] demonstrated the relationship between increased SOX9 and activation of the Hippo pathway in the development of liver fibrosis. Inhibition of YAP1 in CCl_4 and BDL-induced liver fibrosis by injection of specific YAP1-related inhibitor Verteporfin resulted in decreased expression of SOX9 in HSCs. Notch and YAP1 may activate SOX9 in different cell types, both leading to HSCs activation and fibrosis induction [84][85][86]. Noticeably, YAP1/Hippo pathways are related to the activation and amplification of ductular reactive cells (DRC) [87][88]. Additionally, during tissue repair, which normally requires cell proliferation, the Hippo pathway is often downregulated by phosphorylation of its main effectors, yes-associated protein 1 (YAP1) and transcriptional co-activator with PDZ-binding motif (TAZ) [89]. In particular, macrophage-derived TNF-related weak inducer of apoptosis kinase (TWEAK) induced expansion of progenitor cells and proliferation of bile ducts in healthy mice, while fibroblast growth factor–inducible 14 (Fn14) deficient mice or neutralisation of TWEAK prevented the expansion of progenitor cells in cholestasis mice [90][91].

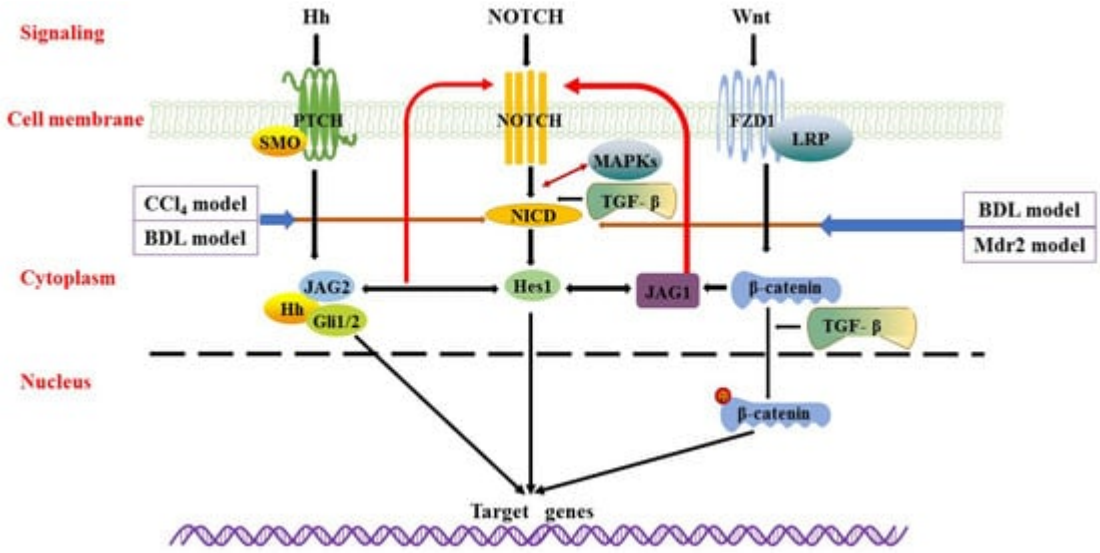

Figure 1. Wnt/β-catenin, Notch, and Hedgehog signalling pathways. Wnt/β-catenin signalling is transmitted through the Frizzled (FZD) receptor, thereby stabilizing β-catenin. Phosphorylated β-catenin translocated into the nucleus to regulate the expression of target genes. There is crosstalk between Wnt/β-catenin signalling and Notch/TGF-β signalling. In Notch signalling, binding of Notch ligands to the receptor results in two proteolytic cleavages to release NICD. The released NICD then translocated into the nucleus, activating transcription factors Hes1, JAG1, and JAG2, whilst the Notch signal pathway interacts with Hh and Wnt signalling pathways. In Hedgehog signalling, Hh ligand secreted by Hedgehog secretory cells binds to PTCH or SMO and generates activated Gli that translocated to the nucleus, inducing target gene expression. These main survival pathways and

sophisticated interactions between signalling pathways (i.e., TGF-β and MAPKs) constitute a complex regulatory network for the survival and proliferation of BECs.

4.2. Hedgehog (Hh) Signalling Pathway

Hh is one of the morphogenetic signalling pathways. There are three mammalian Hh proteins—Sonic hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh). The downstream effector of Hh signalling is Gli1/2, a transcription repressor of the polycomb group and a central regulator of normal stem cell self-renewal. Regardless of the aetiology, it has been proved that the Hh signal is upregulated in the injured liver [92], and it increases with worsening of liver injury and fibrosis [93]. A previous study demonstrated liver accumulation of Hh ligands and activation of the Hh signalling pathway in the livers of BDL rodents and PBC patients [94]. Hh and Notch stimulate each other to promote HSCs activation and subsequent fibrosis [95]. In cholangiocytes, Notch promotes Shh signal transduction by regulating transport inside and outside primary cilium (PC), while Shh promotes Notch signal transduction by directly upregulating Hairy and enhancer of split-1 (Hes1) and Jagged canonical Notch ligand 2 (JAG2) [96] (Figure 2). In vitro and in vivo studies have shown that the Notch signalling pathway drives epithelial to mesenchymal transition (EMT). The interaction between Notch and Hh signalling pathways promotes the transdifferentiation of HSCs into myofibroblasts that involves an EMT, triggering fibrogenesis [76][95]. Finally, Notch also enhances the inflammatory response and M1 polarisation of macrophages [97].

4.3. Wnt Signalling Pathway

The Wnt signalling pathway is likely one of the main factors contributing to the progression of cholestatic liver disease. Wnt signal transduction induces liver fibrosis by promoting HSCs proliferation and activation, accompanied by ECM synthesis, EMT increase, or interaction with other fibrosis mediators [98][99]. Moreover, excessive accumulation of ECM is considered to be a key event in the pathogenesis of ageing-related liver fibrosis [100][101]. Several studies have shown that Wnt signalling is involved in the progression of liver fibrosis, and many components are upregulated and implicated in this process [102][103][104][105] (Figure 1). Conversely, the expression of some Wnt receptors (such as Frizzled 1 (FZD1)) and co-receptor low-density lipoprotein receptor-related protein5/6 (LRP5/6) in activation of HSCs increased in the progression of liver fibrosis but with decreased expression of FZD4/8 [105][106][107][108]. Furthermore, β-catenin is the main downstream effector of classical Wnt signalling, and the loss function of β-catenin will affect the metabolism of BAs. In the BDL model, complete obstruction of bile flow can lead to hepatic cholestasis. Therefore, the enhanced inhibition of BAs synthesis by inhibiting or β-catenin loss can improve the progression of cholestasis. Interestingly, Pradhan [109] showed that the knockdown of β-catenin in $Mdr2^{-/-}$ mice resulted in increased inflammation, cell senescence, promoted fibrosis, and impaired liver regeneration after injury. In $Mdr2^{-/-}$ mice, this phenotype is mainly driven by the toxicity of BAs lacking phospholipids. Therefore, reducing injury, improving regenerative response, and/or maintaining bile flow to prevent stasis are essential for maintaining the liver function, at least in mice.

4.4. Other Related Signalling Pathways

In addition to the above signalling pathways, other studies have found that the c-Jun N-terminal kinase (JNK), a member of the MAPKs family, is involved in the regulation of proliferation, cell death, inflammation, and metabolism [110][111]. JNK contributes to the activation of HSCs, induces overexpression of αSMA during the procession of liver fibrosis [112][113], and promotes the production of myofibroblasts. Kluwe et al. [113] showed that the phosphorylation of JNK increased significantly in mouse liver after BDL or CCl_4 administration, as well as in the human fibrotic liver, mainly in fibroblasts. In vivo, the inhibition using a pan-JNK inhibitor did not affect liver injury but significantly reduced fibrosis after BDL or CCl_4. JNK1-deficient mice showed reduced fibrosis after BDL or CCl_4, while JNK2-deficient mice showed increased fibrosis after BDL but no change after CCl_4. In culture, pan-JNK inhibitors prevent the activation of human HSCs induced by TGF-β, PDGF, and angiotensin II-induced murine HSCs activation, and reduce PDGF and TGF-β signal transduction. Zhao [112] showed the specific role of Jnk1 in HSC activation and ECM formation. The absence of Jnk1 correlated with a lower proliferation and survival of HSCs, demonstrating the pivotal contribution of Jnk1 in the development of liver fibrosis in HSCs. However, signalling pathways involved in JNK activation related to liver fibrosis are also participate in bidirectional crosstalk, including TNF-α and NF-κB [114]. Collectively, sustained activation of pre-fibrosis-related signalling pathways may promote the progression of liver fibrosis, combined with immune cell infiltration around biliary tracts.

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