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Pathophysiological Roles of Ductular Reaction in Liver Inflammation and Hepatic Fibrogenesis

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Abbreviation:

CK = cytokeratin; ECM = extracellular matrix; EMT = epithelial-to-mesenchymal transition; HPCs = hepatic progenitor cells; HSCs = hepatic stellate cells; IL = interleukin; NAFLD = non-alcoholic fatty liver disease; PSC = primary sclerosing cholangitis; SASP = senescence-associated secretory phenotype; TGF = transforming growth factor.

Ductular reaction is referred to as expansion of proliferative and reactive cells that express biliary markers, such as cytokeratin (CK)-7 and CK-19 [1]. Ductular reaction is identified by histological staining in liver specimen of patients with various liver diseases including cholangiopathies, viral hepatitis, and alcoholic and nonalcoholic liver diseases [1]. Ductular reaction is clinically recognized as bile duct hyperplasia; however, the term "ductular reaction" is more commonly used in recent studies to define enhanced intrahepatic bile duct mass that can be attributed to the proliferation of cholangiocytes and/or transdifferentiation of various hepatic cells [1]. It is known that ductular reaction is associated with liver fibrosis and thought to be caused by expansion of hepatic progenitor cells (HPCs) [2]. Accumulating evidence indicates that ductular reaction (i.e., expansion of CK-7^+ or CK-19^+ cells) can be caused not only by HPCs, but also other hepatic cells. Bile duct epithelial cells, cholangiocytes, express CK-7 and CK-19 and proliferate during bile duct injury, which is identified as ductular reaction [1]. Furthermore, in certain pathological conditions, hepatocytes can transdifferentiate into cholangiocyte-like cells expressing CK-7 or CK-19 to compensate the loss of cholangiocyte numbers and functions [3]. Ductular reaction can be induced by expansion of HPCs, cholangiocytes, or hepatocytes, and functional roles of ductular reaction may differ depending on liver diseases or origins of expanding cells. This section will discuss the pathophysiological roles of ductular reaction in liver diseases.

Hepatic cells communicate with each other to coordinate pathophysiological responses against liver injury via secreting cytokines, chemokines, or extracellular vesicles [4]. In the portal area and the HPC niche, HPCs, hepatic stellate cells (HSCs), and macrophages secrete and receive cytokines and growth factors leading to liver inflammation and regeneration [2]. Ductular reaction is associated with portal inflammation and neutrophil infiltration in alcoholic hepatitis, and the cells in ductular reaction (CK-7^+ cells) express elevated neutrophil recruiting chemokines and HPC

markers [5]. In nonalcoholic fatty liver disease (NAFLD), portal inflammation is strongly correlated with liver fibrosis levels and ductular reaction with increased portal infiltration of macrophages and lymphocytes [6]. Ductular reaction and increased portal macrophage infiltration can also be identified in cholestatic liver injury [7]. Cholangiocytes become senescent during cholestatic liver injury, including primary sclerosing cholangitis (PSC) [8]. Senescent cholangiocytes function as a senescence-associated secretory phenotype (SASP) secreting various cytokines, such as interleukin (IL)-6 and transforming growth factor (TGF)-β1 [8]. IL-6 promotes cholangiocyte proliferation and activates macrophages leading to ductular reaction and liver inflammation [9]. These findings indicate the close relationship between ductular reactive cells (i.e., HPCs and cholangiocytes) and portal infiltration and inflammation.

Liver fibrosis is observed in various liver diseases, and the association of expansion of the HPC niche (i.e., ductular reaction) with liver fibrosis has been identified [2]. Liver fibrosis is a hallmark in cholangiopathies, such as PSC, primary biliary cholangitis, and biliary atresia, and ductular reaction is identified along with liver fibrosis [1]. Expansion of CK-7^+ or CK-19^+ cells is found in liver sections of patients with hepatitis B or C virus injection, and ductular reaction levels are correlated with liver fibrosis levels [10]. Ductular reaction levels also correlate with poor prognosis of patients with alcoholic hepatitis and can be used to predict survival rates of patients in severe conditions [11]. A higher grade of ductular reaction determined by histological CK-7 staining is correlated with higher fibrosis stages in NAFLD patients [6]. It is also suggested that ductular reaction is associated with hepatocellular carcinoma. Peritumoral ductular reaction significantly correlates with hepatic inflammation, liver fibrosis, TNM stages, and poor prognosis [12], indicating the association of ductular reaction with the pathophysiology of various liver diseases.

Liver fibrosis is caused by accumulated extracellular matrix (ECM) secreted from activated HSCs or myofibroblasts, major sources of ECM secretion. During PSC, senescent cholangiocytes secrete TGF-β1, which promotes HSC proliferation and activation leading to ECM accumulation and liver fibrosis [13]. Since ductular reaction and liver fibrosis are closely associated and mediated by ductular reactive cells, including cholangiocytes, these cells can be a therapeutic target for liver fibrosis. Inhibition of biliary senescence by p16 Vivo-Morpholino administration downregulated cholangiocyte SASP secretion with attenuated ductular reaction and liver fibrosis in PSC mouse models [14]. Cholangiocytes undergo epithelial-to-mesenchymal transition (EMT) and transform into myofibroblast-like profibrogenic phenotypes secreting robust ECM components contributing to hepatic fibrogenesis in cholestatic liver injury [15]. Inhibition of cholangiocyte EMT by vimentin Vivo-Morpholino attenuated ductular reaction and liver fibrosis in PSC mouse models [15]. Vimentin knockdown also ameliorated liver inflammation and serum TGF-β1 levels in PSC mice [15]. These findings support the close association of ductular reaction and liver fibrosis, and inhibition of ductular reaction can be therapeutic in liver diseases.

Current studies show the pathophysiological roles of ductular reaction and its close association with hepatic inflammation and liver fibrosis in various liver diseases (**Figure 1**). In PSC, inhibiting cholangiocyte senescence or EMT could be a novel therapeutic approach to improve liver conditions by attenuating ductular reaction and liver fibrosis, showing the potentials of cholangiocytes as a promising target of liver diseases. However, not only cholangiocytes but also HPCs and hepatocytes are involved in ductular reaction. The HPC niche is associated with liver fibrosis [2], and elevated HPC marker expression (i.e., expansion of the HPC niche) is associated with neutrophil infiltration and liver inflammation [5]. Hepatocytes transdifferentiate into biliary-like phenotypes during cholestatic liver injury [3]. Although it is not fully elucidated

how HPCs and hepatocytes contribute to the pathophysiology of ductular reaction and liver fibrosis, targeting HPCs and hepatocytes might be another therapeutic approach in liver diseases.

In conclusion, ductular reaction is closely related to liver inflammation and fibrosis and the pathophysiology of liver diseases. Understanding the full dynamic role of ductular reaction during liver injury and identification of potential targets associated with ductular reaction are of paramount significance.

Figure legend

Figure 1. Association of ductular reaction with portal inflammation and liver fibrosis in liver diseases. Expansion of cytokeratin (CK)-7^+ or CK-19^+ cells, which is referred to as ductular reaction, is commonly observed in various liver diseases. The origin of ductular reactive cells includes cholangiocytes, hepatocytes, and hepatic progenitor cells. Ductular reactive cells induce portal infiltration of immune cells, such as macrophages and neutrophils leading to portal inflammation. Ductular reaction is associated with hepatic stellate cell activation leading to secretion of extracellular matrix (ECM) and liver fibrosis. Cholangiocytes transdifferentiate into myofibroblast-like cells contributing to hepatic fibrogenesis via epithelial-to-mesenchymal transition. This figure was created with BioRender (Toronto, Canada).

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Figure 1