Review

DOI: 10.5582/bst.2017.01007

Key role of liver sinusoidal endothelial cells in liver fibrosis

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Summary **Because of the prevalence of viral hepatitis and nonalcoholic fatty liver disease (NAFLD), liver fibrosis has become a very common disease in Asia and elsewhere in the world, constantly increasing the burden of care borne by society. Hepatic sinusoidal capillarization, characterized by gradually shrinking fenestrae on the surface of liver sinusoidal endothelial cells (LSECs) and the formation of an organized basement membrane, is an initial pathologic change associated with liver fibrosis. Basic and clinical studies have indicated that LSECs play a key role in hepatic sinusoidal capillarization by affecting various aspects of the development and progression of liver fibrosis. Reviewing studies on the effect of LSECs on liver fibrosis is essential to better understanding the pathogenesis of liver fibrosis and its mechanism of progression. Moreover, such a review will provide a theoretical basis for identifying new methods to promote the regression or even inhibition of fibrosis. This review will focus on structural and functional changes in LSECs during hepatic sinusoidal capillarization and the interaction between the micro-environment of the liver and the body's immune system.**

Keywords: Liver sinusoidal endothelial cells, capillarization, liver fibrosis, fenestrae, immune system

1. Introduction

Liver fibrosis, characterized by excessive deposition of extracellular matrix (ECM),is a common outcome of chronic liver diseases including viral hepatitis, metabolic diseases, and nonalcoholic fatty liver disease(NAFLD) (*1-3*). Thanks to antivirals and hepatitis B vaccines, liver fibrosis caused by viral hepatitis has decreased in recent years. However, other liver diseases that are prevalent worldwide, such as NAFLD, are gradually becoming key pathogenic factors, particularly in Western countries. Thus, a decline in the incidence of liver fibrosis in the near future seems unlikely and liver fibrosis remains prevalent (*4,5*).Early-stage fibrosis can regress to a nearly normal level when its cause is eliminated; in fact, the self-protective behavior of the body allows it to fight pathogenic factors in that it can limit damage to a particular region (*6-8*). However, advanced fibrosis and cirrhosis can lead to irreversible damage to the liver and eventually cause portal hypertension, bleeding of the digestive tract, and even hepatocellular carcinoma (*2,9*). Hepatic sinusoidal capillarization is a basic pathological change associated with hepatic fibrosis and cirrhosis (*10-13*). Hepatic sinusoidal capillarization plays a key role in the pathogenesis and progression of this process, and it can also induce hepatocellular carcinoma along with a newly forming arterial blood supply (*14-16*). Defenestration of LSECs, a typical phenomenon that occurs during hepatic sinusoidal capillarization, plays a unique role in liver fibrosis and cirrhosis. However, the specific mechanism of capillarization and when defenestration occurs remain unclear.

Released online in J-STAGE as advance publication February 28, 2017.

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2. Role of LSECs in the progression of liver fibrosis/cirrhosis

2.1. *Structure of LSECs and their physiological and pathological function*

LSECs are highly specialized endothelial cells in the human liver. Under normal physiological conditions, LSECs are gateways between hepatocytes and hepatic

sinusoids, they mediate the exchange of plasma, nutrients, lipids, and lipoproteins between hepatic sinusoids and hepatocytes through an ultrafiltration system or the so-called "liver sieve plates", consisting of fenestrae, non-diaphragmed pores that traverse the endothelial cytoplasm (Figures1 and 2) (*17-20*). Generally speaking, the fenestrae are 100-150 nm in size and lack a basement membrane. Their distribution follows some kind of rule, namely larger but fewer fenestrae per sieve plate are seen in the periportal region and smaller but more numerous fenestrae are seen in the centrilobular region (*21,22*). Under some pathological situations, however, their structural and functional features change markedly.

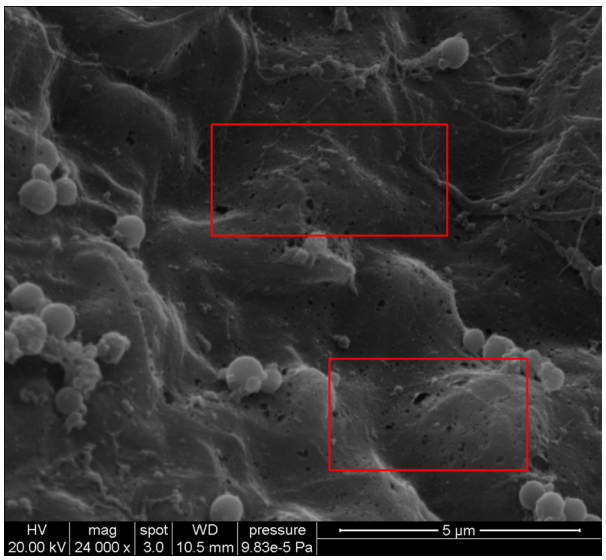

Figure 1. Fenestrae on liver sinusoidal endothelial cells. Numerous fenestrae on the surface of normal LSECs were apparent under a scanning electron microscope.

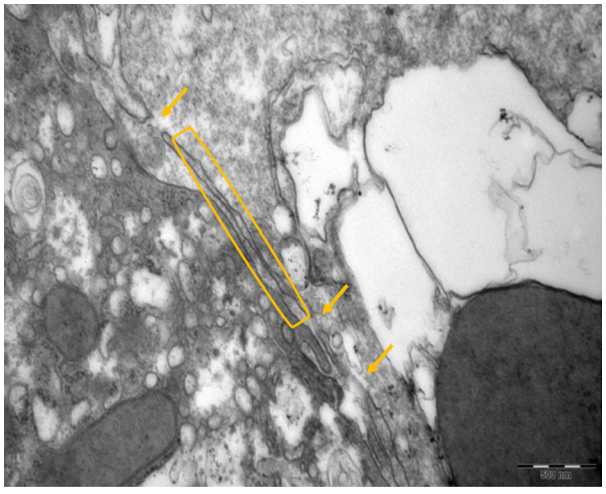

Figure 2. Absence of a basement membrane underneath LSECs. Scattered fenestrae (indicated by yellow arrows) on the surface of normal LSECs were apparent under a transmission electron microscope. This photograph also shows the lack of a basement membrane underneath LSECs (the area enclosed with a yellow line).

2.2. *Capillarization of LSECs*

Capillarization, characterized by defenestration and formation of a continuous basement membrane, is a very common phenomenon in chronic liver diseases. Defenestration is a distinctive structural change during this process. Defenestration accounts for a reduction in the number of fenestrae and fenestrae with a smaller diameter. When capillarization occurs, LSECs actively adjust their structures to adapt to pathogenic factors, such as chronic viral infection and toxins that damage the liver. This change can protect the liver from continuing damage by restricting toxins to a specific area, but it also alters the physiological structure of hepatic sinusoids by promoting the formation of a continuous basement membrane. The bidirectional exchange of molecules between hepatocytes and hepatic blood sinus is disrupted, causing problems such as decreased sinusoidal compliance and increased resistance to blood flow that can affect the physiology of the liver. Disruption of that exchange of molecules may also contribute to the development of portal hypertension during cirrhosis by inducing ischemic atrophy of hepatocytes, leading to increased fibrogenesis and compensatory hypertrophy of surrounding hepatocytes. All of these changes may result in the development of hepatic failure.

3. Structural changes and triggers of defenestration

Thus far, hepatic sinusoidal capillarization has been regarded as a basic pathological change of liver fibrosis, and defenestration of LSECs is viewed as the main characteristic of this pathological change. As described earlier, shrinking fenestrae and formation of a basement membrane are evident during defenestration. However, what initiates this change, in other words what triggers defenestration, has yet to be fully ascertained. Several studies have indicated that aflatoxin, a risk factor for liver cirrhosis and liver cancer, can significantly damage LSECs and reduce their number (*23-25*). A study by Venkatraman *et al*. indicated that the C-terminal fragment of thrombospondin-1 (P4N1), the ligand of CD47, is involved in defenestration of LSECs through the Rho/Rho kinase-myosin signaling pathway (*26*). CD47 can indirectly induce the decrease or even disappearance of LSECs' fenestrae by inhibiting the eNOS-NO-cGMP system (a key signaling pathway during the formation of LSECs' fenestrae) (*27,28*). Additionally, Addo *et al*. indicated that an iron overload in chronic hepatitis could lead to the formation of nerve growth factors that eventually lead to defenestration by binding to TrKA receptors of LSECs (*29*). Although there are differing opinions on the factors that influence LSECs' defenestration, the exact mechanism remains unknown and needs to be examined further.

4. LSECs and their microenvironment

Liver fibrosis is a gradual process that affects the hepatic parenchyma and its microenvironment. In general, the defenestration of LSECs occurs earlier than the formation of fibrous septa. After fibrous septa form, abasement membrane begins to appear. This process indicates that defenestration may be the starting point for liver injury, and the formation of a sub-endothelial basement membrane could be the result of the deposition of ECM during liver fibrosis. By creating a barrier between the hepatic sinusoids and hepatocytes, hepatic sinusoid capillarization decreases the exchange of oxygen and nutrients in hepatic cells, thus worsening damage to the liver. ECM continues to be deposited in Disse's space as the process of hepatic fibrosis progresses. This vicious cycle of liver damage eventually leads to hepatic atrophy and collapse of hepatic sinusoids.

4.1. *LSECs and hepatic stellate cells*

Hepatic stellate cells (HSCs) are one type of nonparenchymal cells of the liver that are close to sinusoids in Disse's space. Since HSCs store retinoids and produce glial fibrillary acidic protein (GFAP), they are also called fat-storing cells or vitamin A-rich cells (*30-33*). Generally speaking, activated-HSCs are known to be a key factor in fibrogenesis. When pathologic liver injury occurs, HSCs convert cellular phenotypes from a quiescent to an activated myofibroblastic state and cause liver fibrosis by secreting fibrogenic cytokines, including tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), platelet-derived growth factor (PDGF), and transforming growth factor β (TGF-β) (*34,35*).Normally, LSECs can keep HSCs quiescent through nitric oxide (NO) production stimulated by vascular endothelial growth factor (VEGF) (*36*). However, defenestration and capillarization of LSECs due to liver injury promotes the activation of HSCs, thereby inducing liver fibrosis through loss of VEGF-stimulated NO production. Studies have indicated that LSECs in different states of differentiation affect HSCs differently. That is, differentiated LSECs promote the quiescence of HSCs and they accelerate the regression and prevent the progression of fibrosis, while capillarized LSECs do the opposite (*12,22,36*).

4.2. *LSECs and macrophages*

Hepatic macrophages are important to the pathogenesis of chronic liver injury. The general consensus is that hepatic macrophages can either arise from circulating monocytes or from self-renewing embryo-derived local macrophages, which are also called Kupffer cells (KCs). KCs are usually considered to be a key factor for the initiation and progression of fibrosis. When the human body is subjected to harmful influences such as viral hepatitis and alcohol consumption, KCs can be induced into an activated state in which they secrete a wide variety of proinflammatory cytokines, such as IL-6, IL-10, IL-13, TNF-α, and TGF-β, further activating HSCs. Macrophages can play an indirect role in the development of liver fibrosis (*37-40*). You *et al*. found that KCs can substantially affect liver blood vessel repair by expressing various angiogenic factors and inducing the proliferation and migration of LSECs, thereby accelerating tissue recovery from acute injury (*41*). However, the correlation between LSECs and macrophages in chronic liver diseases and the mechanism of their interaction remains unclear and needs to be examined further.

4.3. *LSECs and T lymphocytes*

There are many classifications of T lymphocytes. In accordance with the differential markers of T cells, T lymphocytes can be divided into two groups, namely $CD4^+$ T cells and $CD8^+$ T cells. Depending on the different functions of T cells in the immune response, T lymphocytes can be divided into helper T lymphocytes (Th cells), cytotoxic T lymphocytes (CTL, or Tc cells), and regulatory T cells (Tr cells). Various T cells have different effects during the progression of chronic liver diseases. Several studies have indicated that $CD8^+$ T and $CD4^+$ T lymphocytes are recruited within the liver in ALD and NAFLD and that T lymphocytes are associated with the prolonging of intralobular inflammation, piecemeal necrosis, and septal fibrosis (*42-45*). In additional, Th cells, and especially the conventional Th1 and Th2 subtypes, also play a key role in fibrosis. Under normal conditions, Th1 and Th2 lymphocytes maintain a dynamic balance that helps to facilitate the body's immune response. When the liver is exposed to infection or toxins for a prolonged period, this balance is disrupted. Studies have indicated that Th2 lymphocytes stimulate the development of hepatic fibrosis after liver injury while Th1 lymphocytes do the opposite (*46-50*). A study by Bonder *et al*. found that Th1 and Th2 cells respectively recruit and use α4β1-integrin and vascular adhesion protein (VAP)-1,two cell surface molecules expressed on LSECs, to adhere to liver sinusoids during liver fibrosis (*51*) (Figure 3).In addition, LSECs can be activated, express adhesion molecules, and synthesize chemokines that are exposed on their luminal surface during inflammation whileVAP-1 can be upregulated by chronic inflammation (*52-55*). However, the relationship between T cells and defenestration of LSECs during hepatic sinusoid capillarization still needs to be explained further.

5. Liver fibrosis and immunoregulation

The anatomical organization of the liver is crucial to its immune functions. As everyone knows, the liver

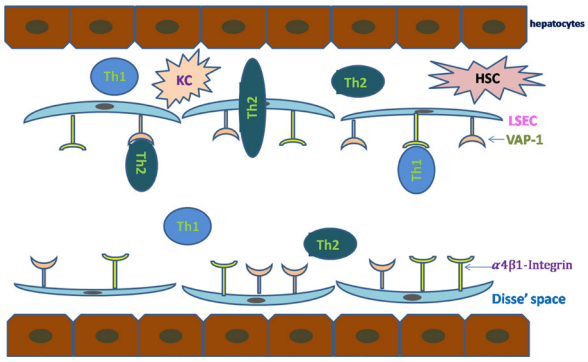

Figure 3. Diagram of interaction between Th cells and liver sinusoidal endothelial cells. Note that Th1 and Th2 cells respectively recruit using α4β1-integrin and vascular adhesion protein-1 and adhere to liver sinusoids during liver fibrosis. HSC, hepatic stellate cells; KC, Kupffer cells; LSEC, liver sinusoidal endothelial cells; Th1/Th2, helper T lymphocytes; VAP-1, vascular adhesion protein-1.

has two blood supply systems, an arterial system and a portal vein system. Ordinarily, about 30% of all blood passes through the liver per minute, carrying about 108 peripheral blood lymphocytes a day (*56,57*). Because of the unique structure of LSECs, infiltrating lymphocytes can penetrate into Disse's space and even enter hepatocytes by crossing scattered fenestrae; this behavior allows immune cells in the liver to function (*58*). Currently, an innate immune response is viewed as the main factor for the onset of hepatic inflammation in both alcoholic steatohepatitis (ASH) and nonalcoholic steatohepatitis (NASH) (*59*).During HBV-related liver fibrosis, however, the homeostasis of $CD4^+$ T cells is pivotal (*60*).Another critical balance that has attracted increasing attention involves helper T17 (Th17) cells and Tr cells. Several studies have reported that IL-17,a pro-inflammatory factor mainly secreted by Th17, is correlated with the severity of liver diseases(*61,62*). The severity of liver fibrosis is also closely related to the number of Th17 cells (*63,64*). Tr cells are thought to limit liver fibrosis by inhibiting HSC activation and proliferation (*65,66*). Nevertheless, the underlying mechanisms regulating the Tr/Th17 balance during liver fibrosis have yet to be fully explained.

6. Conclusion

Thanks to the use of hepatitis B vaccines, hepatitis B-related liver fibrosis will decrease, but liver fibrosis will remain a serious problem because of the prevalence of NAFLD. Hepatic sinusoidal capillarization is a basic pathological feature of hepatic fibrosis and cirrhosis, and defenestration of LSECs is garnering increasing attention as an essential characteristic of that capillarization. However, the exact mechanism of defenestration, the interaction between LSECs and other interstitial cells, and the role of immune regulation in the progression of liver fibrosis still need to be explained further, so substantial work needs to be done in the future.

Acknowledgements

This project was supported by the National Natural Science Fund of China (81470860, Yuesi Zhong), the Science and Technology Planning Project of Guangdong Province, China (2014A020212575, Yong Zou), and Natural Science Foundation of Guangdong Province, China (2016A030313357, Yong Zou).

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(Received January 10, 2017; Revised February 16, 2017; Accepted February 23, 2017)