Perspective

Hypoxia-inducible factor may induce the development of liver fibrosis in Budd–Chiari syndrome by regulating CD248/endosialin expression: A hypothesis

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ABSTRACT

Budd–Chiari syndrome (BCS) leads to the development of liver fibrosis in most of the cases. However, the mechanism of BCS-related liver fibrosis is unclear, and it may be largely different from that induced by chronic viral hepatitis. Hepatic stellate cell (HSC) and its specific marker CD248/endosialin are known to play an important regulatory role in the development of liver fibrosis. Additionally, hypoxia microenvironment and hypoxia-inducible factor (HIF) are involved in the regulation of CD248/endosialin. Therefore, we hypothesize that hypoxia microenvironment which develops due to BCS can regulate the expression of CD248/endosialin in HSC via HIF signaling pathway, which then affects the function of HSC and development of liver fibrosis. To confirm the hypothesis, two major investigations are necessary: (1) in the BCS animal model and clinical studies, the relationship between the severity of liver fibrosis and the expression of HIF and CD248/endosialin in HSC will be explored; and (2) in the *in vitro* cell system, the effect of hypoxic microenvironment, HIF-1α or HIF-2α, on the expression of CD248/endosialin in HSC will be explored. It will be important to elucidate whether HIF signaling pathway regulates the expression of CD248/endosialin, thereby inducing the development of BCS-related liver fibrosis.

Key words: Budd–Chiari syndrome, liver fibrosis, hepatic stellate cell, CD248/endosialin, hypoxia-inducible factor

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BACKGROUND

Budd–Chiari syndrome

Budd–Chiari syndrome (BCS) is a vascular disorder of the liver, characterized by hepatic venous outflow obstruction from the major hepatic vein to the confluence between suprahepatic portion of inferior vena cava and right atrium.[1-3] According to the epidemiological data from Denmark[4], France[5], Korea[6], northwestern Italy[7], and Sweden[8], the annual incidence of BCS ranges from 0.13 to 3.5 per million in the mentioned countries. Epidemiological data from Japan[9] and Sweden[8] reveal that the prevalence of BCS in those countries is between 1.4 and 4.02 per million. Acute or fluminant BCS can result in acute life-threatening liver failure. Chronic BCS can progress to liver fibrosis and cirrhosis, resulting in cirrhosis-related complications, such as ascites and variceal bleeding[10-11], and even hepatocellular carcinoma.[12-13] Histologically, veno-portal cirrhosis has been found to be the major pattern of fibrosis developing in some common liver diseases, such as viral hepatitis and alcohol abuse.[14,15] By comparison, the histological patterns of liver fibrosis in BCS are different from those in common chronic liver diseases. Veno-centric cirrhosis, also called as reversed nodulation cirrhosis, is more

specific to BCS and is characterized by bridging fibrosis between hepatic veins without septa to portal tracts.[15]

CD248/endosialin

Searching for "CD248" or "endosialin" in the PubMed database, we retrieved a total of 108 relevant papers. In the first paper published in 1992[16], Rettig *et al.* identified the expression of endosialin for the first time in the vascular endothelial cells of human malignant tumors, including sarcomas, carcinomas, and neuroectodermal tumors. So CD248/endosialin is also called as a tumor endothelial marker 1[17]. Recently, a monoclonal antibody to endosialin, also called as MORAb-004 or ontuxizumab, has been developed and its safety in the treatment of advanced solid tumors has been explored in a phase I clinical trial.[18] More recently, two phase II clinical trials from US for the use of ontuxizumab have been published.[19,20] The first phase II trial randomized the patients with chemorefractory metastatic colorectal cancer into ontuxizumab (8 mg/kg) or placebo plus best supportive care groups. The second phase II trial randomized the patients with metastatic melanoma receiving at least one prior systemic treatment but developing disease progression into ontuxizumab (2 mg/kg) or ontuxizumab (4 mg/kg) groups. These preliminary findings suggested the potential antitumor efficacy of ontuxizumab by inhibiting CD248/endosialin.

Association of CD248/endosialin with hepatic stellate cell and liver fibrosis

In addition to the expression of CD248/endosialin in tumors, some studies also suggested its expression in liver fibrosis. In qRT-PCR analyses performed by Mogler and colleagues[21], a weak expression of CD248/endosialin was restricted to hepatic stellate cells and liver sinusoidal endothelial cells in healthy adult murine, but CD248/endosialin expression was not observed in Kupffer cells or hepatocytes. Similarly, a weak expression of CD248/endosialin was restricted to hepatic stellate cells in healthy adult human livers, but CD248/endosialin expression was not observed in hepatocytes. Therefore, CD248/endosialin might be a specific marker for hepatic stellate cells. Furthermore, CD248/endosialin expression was significantly higher in human fibrotic and cirrhotic liver than in normal human liver. In another study, Wihelm and colleagues measured the expression of CD248/endosialin at gene and protein levels in human and mouse liver tissues.[22] qPCR analyses also showed that the expression of CD248/endosialin mRNA were higher in cirrhotic patients due to alcohol abuse, non-alcoholic steatohepatitis, and autoimmune-related liver diseases than healthy controls. Similarly, the expression of CD248/endosialin mRNA was higher in carbon tetrachloride-induced liver fibrosis mice than in normal mice. In addition, immunofluorescent staining analysis demonstrated that the number of $CD248^+$ cells was higher in cirrhotic patient liver tissues than in healthy human liver tissues. Similarly, the number of $CD248^+$ cells was also higher in carbon tetrachloride-induced liver fibrosis mouse liver tissues than in normal mouse liver tissues. Taken together, both studies suggested a close relationship between CD248/endosialin and development of liver fibrosis.

Hypoxia and hypoxia-inducible factor (HIF)

The critical role of hypoxia and HIF in tumor development, progression, metastasis, and prognosis has been widely recognized.[23-25] Recently, our group also reviewed the evidence regarding the association of expression of HIFs and gene polymorphism with risk, stage, and survival of various human cancers.[26] Furthermore, HIFs could be involved in the development of various liver diseases.[27-28] Targeting HIFs may be promising for the treatment of liver diseases.

Association of hypoxia/HIF with hepatic stellate cell and liver fibrosis

Experimental evidence supports the association of hypoxia with liver fibrosis. Corpechot *et al.* found that the level of collagen I, a marker of fibrosis, in activated hepatic stellate cells was increased under hypoxic conditions, but not the level of collagen IV.[29] Additionally, the expression of pimonidazole adducts in the hepatocytes, a marker of cellular hypoxia, was significantly higher in the rats with cirrhotic or fibrotic livers than those with normal livers. In another experimental study, Moon *et al.* found that the proportion of hypoxia area, which was characterized by the expression of pimonidazole–sulfur adducts, was gradually increased with the time of bile duct ligation in mice.[30] HIF-1 alpha levels were also increased with the time of bile duct ligation in mice.

On the other hand, experimental studies suggested that the severity of liver fibrosis should be decreased in HIF-1-alpha-deficient mice. Moon *et al.* showed that the collagen I mRNA expression level was significantly lower in HIF-1-alpha-deficient mice subjected to bile duct ligation than control mice subjected to bile duct ligation. Similarly, Copple *et al.* demonstrated that the expression of collagen I mRNA was significantly increased in wild-type mice after bile duct ligation, but decreased in HIF-1-alpha-deficient mice after bile duct ligation.[31] Roychowdhury *et al.* provided moderate chronic ethanol feeding to the carbon tetrachloride–induced mice and found that the area of Sirius Red staining was lower in hepatocyte-specific HIF-1-alpha-deficient mice.[32]

Association of CD248/endosialin with hypoxia/HIF

Only one experimental study by Ohradanova *et al.* investigated the regulatory role of hypoxia on endosialin expression. It showed that CD248/endosialin mRNA and protein levels in human cell lines were significantly higher in hypoxic conditions than normoxic conditions.[33] HIF-2 alpha binds hypoxia-responsive element at position –976/–969, thereby regulating the endosialin promoter and hypoxic induction of endosialin.

HYPOTHESIS

Based on the existing knowledge, we hypothesize that hypoxia microenvironment induced by BCS can regulate the expression of CD248/endosialin in HSC via HIF signaling pathway, which then affects the function of HSC, leading to the development of liver fibrosis.

EVALUATION OF HYPOTHESIS

At least two major investigations are necessary to evaluate the hypothesis. In the first investigation, we will conduct the BCS animal model and clinical studies to explore the relationship between the severity of liver fibrosis and the expression of HIF and CD248/endosialin in HSC. In the second investigation, we will conduct the *in vitro* cell experiments to explore the effect of hypoxic microenvironment, HIF-1α or HIF-2α, on the expression of CD248/endosialin in HSC. Besides, we will explore the potential regulatory mechanisms of CD248/endosialin. For example, we may explore the effect of endosialin on the MAP kinase ERK-1/2 phosphorylation and early transcription factor c-Fos.

CONCLUSIONS

To our knowledge, few experimental studies have been performed regarding the mechanisms of liver fibrosis in BCS. Therefore, this hypothesis is important to elucidate whether HIF signaling pathway regulates endosialin expression, thereby inducing the development of BCS-related liver fibrosis.

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Conflict of Interest

None.

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