Histol Histopathol (2013) 28: 145-154
DOI: 10.14670/HH-28.145

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Role of endothelial-mesenchymal transition in idiopathic portal hypertension

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Summary. Idiopathic portal hypertension (IPH) is a condition of non-cirrhotic portal hypertension without a known cause of liver disease. Obliterative portal venopathy is regarded as the primary lesion, which is responsible for the pre-sinusoidal block of hepatic blood flow leading to the development of IPH. The disease pathogenesis of IPH seems to be heterogeneous, and the pathogenic mechanisms of obliterative portal venopathy have not been fully understood. Owing to the limited understanding of the disease pathogenesis, the treatment of IPH is still largely supportive. Recently, endothelial dysfunction has been documented during the development of portal hypertension, and its contribution to IPH is being analyzed. Endothelial-mesenchymal transition (EndMT) is a phenomenon whereby vascular endothelial cells acquire myofibroblastic features characterized by an ability to express mesenchymal cell products that are related to tissue fibrogenesis. In addition to cardiovascular development, there is increasing evidence showing that EndMT is likely to be involved in a variety of fibrotic diseases, such as cardiac, pulmonary, and renal fibrosis. This article reviews the recent progress in studies of the pathogenic mechanisms of IPH in terms of endothelial dysfunction of portal veins. In particular, the role of EndMT in obliterative portal venopathy of IPH is highlighted and discussed.

Key words: Idiopathic portal hypertension, Endothelial-mesenchymal transition, Obliterative portal venopathy, Hepatoportal sclerosis, Fibroelastosis

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Introduction

Idiopathic portal hypertension (IPH) is defined as a condition of non-cirrhotic portal hypertension in the absence of a known cause of liver disease and with a patent extrahepatic portal vein (Nakanuma et al., 2001; Schouten et al., 2011). It is also referred to as hepatoportal sclerosis and non-cirrhotic portal fibrosis, although there have been several differences in the application of these terms. Portal hypertension in patients with IPH arises because of pre-sinusoidal obstruction of hepatic blood flow. Obliterative venopathy with luminal narrowing or obliteration of small portal veins accompanied by dense deposits of elastic fibers is one of the most characteristic findings in the liver histology of IPH, which is responsible for the pre-sinusoidal block of hepatic blood flow (Nakanuma et al., 1996; Chawla and Dhiman, 2008; Cazals-Hatem et al., 2011).

IPH is considered a disorder with a relatively benign disease course, but progression to liver failure, hepatic encephalopathy, and hepatopulmonary syndrome has been encountered (Sawada et al., 2007; Eapen et al., 2011). In a proportion of cases, indications for liver transplantation are considered (Bernard et al., 1995; Krasinskas et al., 2005; Isabel Fiel et al., 2007). Many etiological factors related to the development of IPH have been proposed, including immunological disorders, chronic infections, exposure to medication or toxins, genetic disorders, and thrombophilia (Schouten et al.,

Abbreviations: AECA, anti-endothelial cell antibodies; α-SMA, α-smooth muscle actin; BMP, bone morphogenic protein; CTGF, connective tissue growth factor; EMT, epithelial-mesenchymal transition; EndMT, endothelial-mesenchymal transition; HMVEC, human dermal microvascular endothelial cell; IPH, idiopathic portal hypertension; NOS, nitric oxide synthase; SSc, systemic sclerosis; TGF-ß, transforming growth factor-ß; T,R-I, TGF-, receptor type I; T,R-II, TGF-ß receptor type II; VCAM-1, vascular cell adhesion molecule-1

2011). The disease pathogenesis of IPH seems to be heterogeneous, and the pathogenic mechanisms of obliterative portal venopathy have not been fully understood. Owing to the limited understanding of the disease pathogenesis, treatment of IPH remains largely supportive and is directed toward treating complications of portal hypertension (mainly variceal bleeding and splenomegaly) (de Franchis, 2010).

Recently, endothelial dysfunction has been documented during the development of portal hypertension, as well as liver cirrhosis, and its contribution to IPH is being analyzed (Iwakiri, 2012). This article reviews the recent progress in studies of the pathogenic mechanisms of IPH in terms of endothelial dysfunction of portal veins, particularly focusing on the role of endothelial-mesenchymal transition (EndMT) in obliterative portal venopathy. First, the pathological features of IPH are briefly described to obtain a better understanding of the disease pathogenesis.

Pathological features of IPH

Gross pathology

Macroscopic features in IPH may vary according to the extent of disease progression, and they have been examined mainly in livers obtained from pathological autopsies and liver transplantation. The majority of these livers show atrophy and dysmorphy, and old occluding or mural thrombi are frequently seen in the large portal vein branches (Nakanuma et al., 2001; Sawada et al., 2007). In some patients, gross appearance is normal, and nodular appearance and dilatation of the portal vein trunk can also be encountered (Nakanuma et al., 2001).

Histopathology

The pathological features of IPH livers are not pathognomonic. Frequently observed histological features include obliterative portal venopathy of small portal veins, periportal/perisinusoidal fibrosis, and dilated portal veins herniating into the surrounding parenchyma (Nakanuma et al., 2001; Okudaira et al., 2002; Tsuneyama et al., 2003). Obliterative portal venopathy is generally regarded as the primary lesion accounting for the development of IPH (Cazals-Hatem et al., 2011).

Portal tracts often show collagen deposition, and dense deposition of elastic fibers accompanied by obliterated small portal vein is also a characteristic finding (Fig. 1). They are irregularly distributed in an individual liver. Portal tracts are variably enlarged, and occasionally show thin fibrous septa growing into the hepatic parenchyma. Portal inflammation is typically negligible. In some patients, hepatic fibrosis corresponds to incomplete septal cirrhosis (Roskams et al., 2003; Hübscher, 2011). The fibrogenic process may be mediated partially by activated hepatic stellate cells (Tsuneyama et al., 2002).

Hilar and intrahepatic large and medium-sized portal veins are open or even dilated in established IPH livers. Phlebosclerosis is a common finding, and the major portal vein branches show fibroelastosis and intimal thickening to various degrees (Fig. 2). In the advanced disease stage, they are occasionally occluded by old thrombi with recanalization.

Atrophy of hepatic parenchyma, which is accentuated in the subcapsular region, is often observed during disease progression. Such cases are preferentially observed in perivenular areas and between hyperplastic hepatic nodules. Hepatocytes are more or less atrophic or small, and apoptosis of hepatocytes is occasionally seen (Tsuneyama et al., 2002). In some cases, the liver histology is almost normal, except for portal venous dilatation or obliteration. Hyperplastic lesions such as nodular regenerative hyperplasia and partial nodular

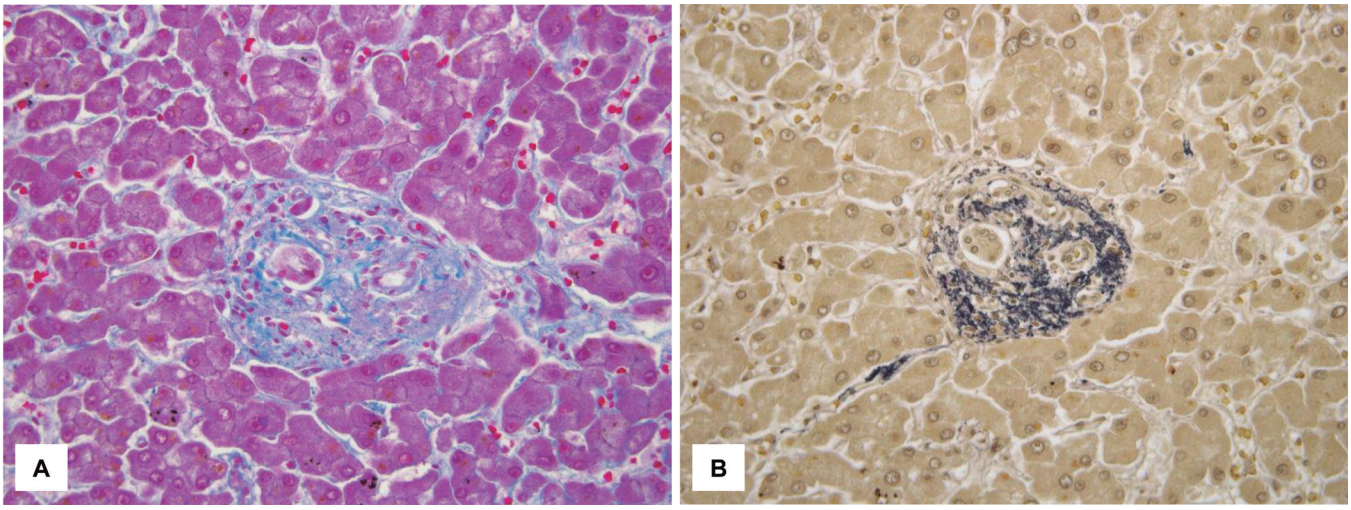


Fig. 1. Fibrotic portal tract of IPH with obliterated small portal vein. **A,** Azan-Mallory staining; **B,** Elastica-van Gieson staining.

transformation are encountered in a proportion of cases, which may result from disturbed intrahepatic circulation (Wanless, 1990; Nakanuma et al., 1996).

EndMT

General aspects

During the development and progression of pathological fibrosis, myofibroblasts play an important role in the production of extracellular matrix molecules. Myofibroblasts are derived from at least three sources: resident tissue fibroblasts, transition of epithelial cells into mesenchymal cells (epithelial-mesenchymal transition, EMT), and bone marrow-derived circulating fibrocytes (Wynn, 2008).

Recently, EndMT has emerged as another possible source of tissue myofibroblasts (Piera-Velazquez et al., 2011; Kovacic et al., 2012). EndMT is a complex biological process that is recognized as cellular transdifferentiation characterized by the down-regulation of vascular endothelial markers such as CD31 and von Willebrand factor, and the emergence of myofibroblastic markers such as S100A4/fibroblast-specific protein-1 and α-smooth muscle actin (α-SMA). Endothelial cells that undergo EndMT acquire an ability to express mesenchymal cell products such as type I collagen, thereby contributing to the fibrotic process.

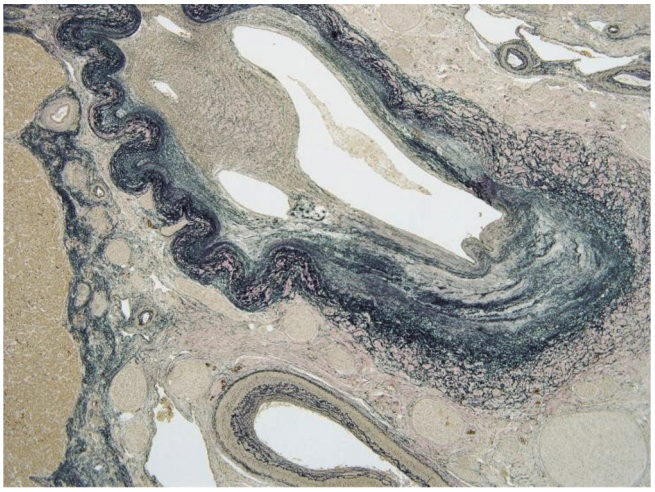


Fig. 2. Hilar portal vein of IPH with fibroelastosis and an organized thrombus. Elastica-van Gieson staining.

Associated conditions and diseases

The essential role of EndMT in cardiovascular development has been thoroughly studied (Kovacic et al., 2012). Recently, increasing evidence has been accumulated showing that EndMT is likely to be involved in a variety of fibrotic diseases. The involvement of EndMT has been shown in cardiac, pulmonary, and renal fibrosis (Zeisberg et al., 2007). In corneal injury, acute inflammatory response following the injury is regarded as important in the induction of EndMT (Lee et al., 2012). The role of inflammation-induced EndMT has also been investigated in intestinal fibrosis, indicating its role in chronic inflammatory diseases such as inflammatory bowel disease (Rieder et al., 2011). Carcinoma-associated interstitial fibrosis has been shown to be mediated by EndMT (Zeisberg et al., 2007).

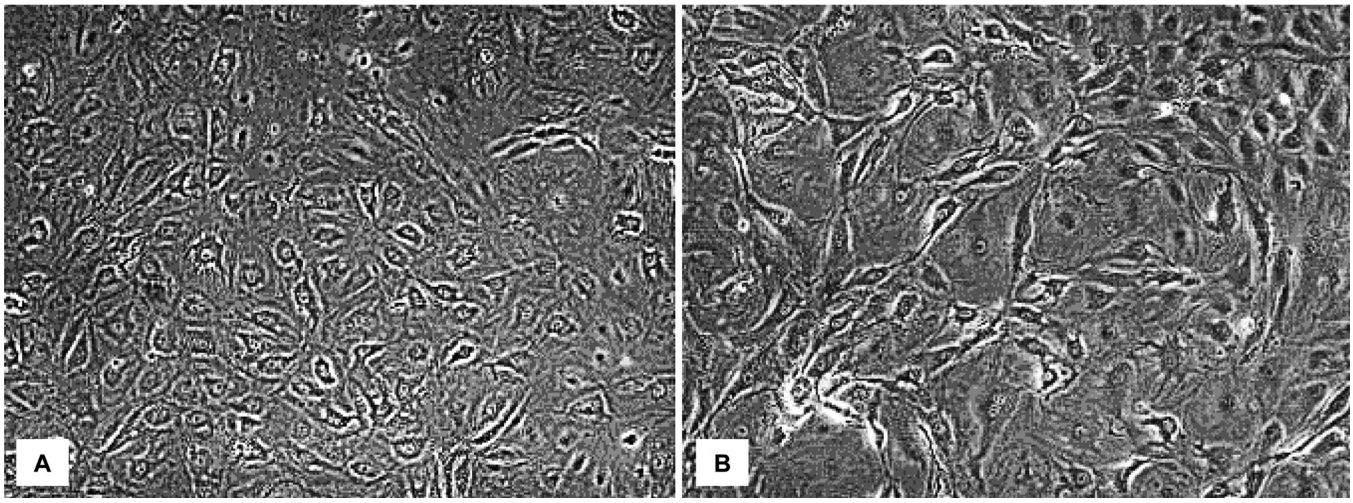


Fig. 3. Morphological alteration of human dermal microvascular endothelial cells (HMVEC) by TGF-ß1. HMVEC grow in the form of an epithelioid, sheet-like appearance **(A),** and treatment with TGF-ß1 changes the cellular morphology from an epithelioid to a spindle-shaped appearance **(B)**. Phase-contrast microscopy.

Systemic sclerosis (SSc) is another example in which the process of EndMT may be involved in the disease pathogenesis (Karasek, 2007). Of relevance, SSc bears many of the hallmarks of conditions involving EndMT, including mesenchymal cell proliferation and TGF-ß signaling. Although a chronic inflammatory condition affecting the vasculature is considered to be related to the occurrence of EndMT (Chaudhuri et al., 2007), an established lesion of dense cutaneous fibrosis in SSc usually lacks significant inflammatory cell infiltrates, which is similar to the histological lesion of hepatoportal sclerosis of IPH.

In the liver, the fibrogenic process is mediated by myofibroblasts in most types of chronic liver disease, which originate from hepatic stellate cells. Fibrogenic liver injury is often accompanied by portal and/or parenchymal inflammation. Because α-SMA-positive activated hepatic stellate cells are focally found in the perisinusoidal area of IPH livers, parenchymal fibrosis may be explained by the contribution of myofibroblasts (Tsuneyama et al., 2002). However, α-SMA-positive myofibroblast-like cells are rarely seen in the peripheral portal tracts of IPH, suggesting that other matrix-producing cells may exist in the portal tracts.

In addition to residual portal fibroblasts, EndMT may contribute to portal fibrosis of IPH (Kitao et al., 2009), although the role of EndMT in hepatic fibrogenesis has not been fully studied. The contribution of bone marrow-derived fibrocytes, as well as the process of EMT of hepatocytes and biliary epithelial cells, is still under debate (Higashiyama et al., 2009; Popov and Schuppan, 2010; Wells, 2010; Scholten et al., 2011).

Molecular mechanisms

Transforming growth factor-ß (TGF-ß) plays a crucial role in tissue fibrosis and is implicated in the pathogenesis of numerous disorders. Similar to EMT, TGF-ß acts as a potent inducer of EndMT both *in vitro* and *in vivo*. In fact, human dermal microvascular endothelial cells (HMVEC) undergo morphological alteration from an epithelioid, sheet-like appearance into a spindle-shaped, myofibroblastic-like appearance following TGF-ß1 treatment *in vitro* (Fig. 3). The spindle-shaped HMVEC following TGF-ß1 treatment has been shown to exhibit reduced expression of a vascular endothelial cell marker, CD34, and increased expression of S100A4, α-SMA, COL1A1, and pSmad2 (Kitao et al., 2009).

During cardiovascular development, TGF-ß2, TGF-ß3, and bone morphogenic proteins 2 and 4 (BMP-2 and -4) are required for initiation and completion of EndMT (Armstrong and Bischoff, 2004). Crucial roles of signaling pathways, including wnt/ß-catenin and Notch, have been suggested in the process (Gitler et al., 2003; Luna-Zurita et al., 2010). Endocardial EndMT is also dependent on receptor tyrosine kinase signaling via the phospho-inositide-3 kinase-phosphoinositide-dependent protein kinase 1-Akt/protein kinase B cascade, which is upstream of Snail (Kovacic et al., 2012). Recently, differential expression or microRNA has been analyzed during cardiac EndMT (Ghosh et al., 2012).

TGF-ß binds to TGF-ß receptor type II (TßR-II), and recruits the TGF-ß receptor type I (TßR-I). TßR-I subsequently phosphorylates Smad2 and Smad3, and they translocate from the cytoplasm to the nucleus, where they regulate transcription of target genes. BMP-7 is a member of the TGF-ß superfamily, and a promising TGF-ß antagonist by counteracting Smad2/3 phosphorylation (Kinoshita et al., 2007). Indeed, it has been shown that BMP-7 inhibits the TGF-ß1-induced EndMT in HMVEC *in vitro*, and it was also shown to abrogate EndMT induced by TGF-ß1 in a mouse model

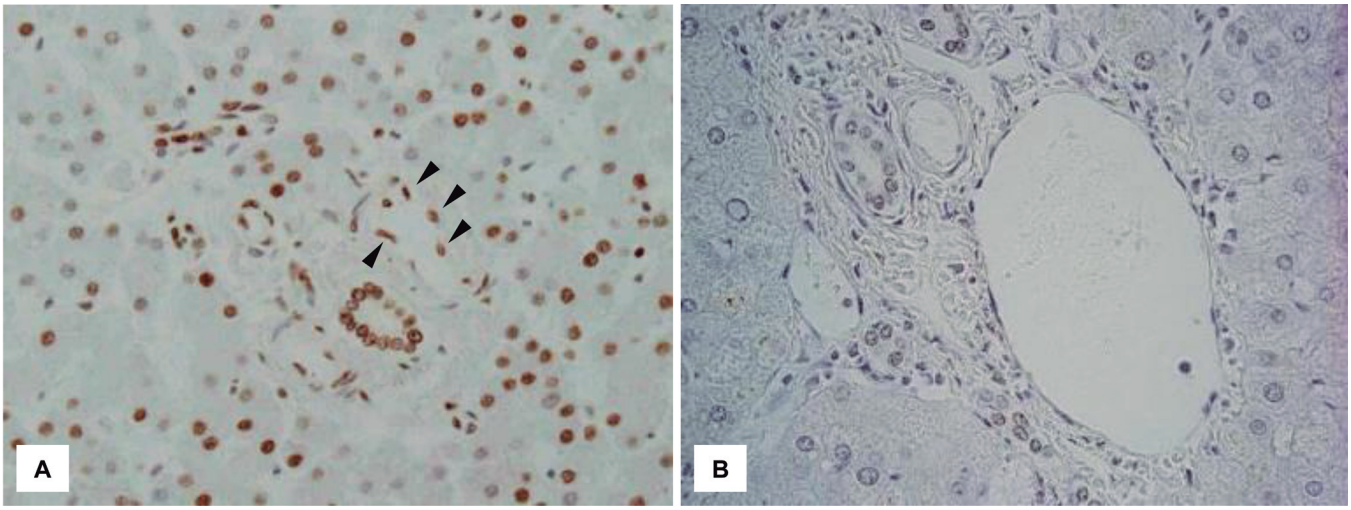


Fig. 4. Increased immunohistochemical expression of pSmad2 in IPH liver. Arrowheads indicate pSmad2-positive endothelial cells of peripheral portal tract of IPH. **A.** IPH. **B.** Normal liver.

of cardiac fibrosis *in vivo* (Zeisberg et al., 2007). In addition, the blockade of EndMT by a Smad3 inhibitor was found to delay the development of diabetic nephropathy in a mouse model (Li et al., 2010).

EndMT also occurs independently of Smad2/3 activation. The non-Smad pathway of TGF-ß signaling involves the participation of several kinases, including the c-Abl protein kinases, protein kinase Cδ, and glycogen synthase kinase 3ß, and the kinase inhibitor molecules have been suggested to be effective therapeutic agents for SSc (Li et al., 2011). Inflammatory cytokines, such as interleukin-1ß, also play a role in the initiation of EndMT. Importantly, EndMT may be a reversible process (Arciniegas et al., 2007), and therefore represents a novel therapeutic target for fibrotic disorders.

EndMT in IPH

Overlap of IPH and SSc

IPH has frequently been reported in association with immunological disorders, including SSc, systemic lupus erythematosus, rheumatoid arthritis, mixed connective tissue disease, and celiac disease (Tsuneyama et al., 2002; Schouten et al., 2011). Various theories have been proposed to explain this.

SSc is a disease that causes excessive collagen production and deposition, vascular damage, and inflammation in multiple organs including skin, lung, and the gastrointestinal tract. Patients with the disease show increased deposition of collagen types I and III in various organs, with type I being the most abundant (Charles et al., 2006). Given the etiological fact that SSc is a clinical complication of IPH, it is possible that similar pathogenic mechanisms of collagen deposition exist between IPH and SSc (Nakanuma et al., 2009). Because EndMT is believed to be involved in excessive collagen deposition in patients with SSc, it may also be related to the portal fibrosis of IPH. In the following sections, the role of EndMT in obliterative portal venopathy of IPH is discussed on the basis of the results of our study (Kitao et al., 2009).

EndMT in IPH liver

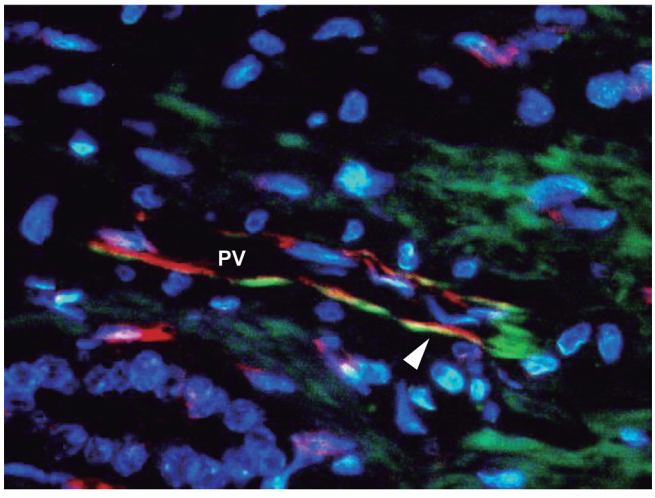
Many IPH livers show diffuse and strong nuclear expression of pSmad2 throughout the liver, including portal vein endothelium, hepatocytes, and biliary epithelial cells (Fig. 4A). In contrast, positive immunohistochemical expression of pSmad2 is rarely seen in sections of normal liver (Fig. 4B). Since both TßR-I and TßR-II are diffusely expressed in the liver, including portal vein endothelium of IPH as well as normal liver, the increased expression of pSmad2 in the IPH livers may reflect the activation of the signaling pathways, including TGF-ß, leading to the occurrence of EndMT in the endothelium.

Diffuse and strong immunoexpression of pSmad2 in hepatocytes, as well as portal vein endothelium in IPH livers, also indicates that hepatic parenchymal atrophy frequently seen in IPH patients at an advanced disease stage may be associated with the growth inhibitory effects of TGF-ß on hepatocytes, as well as the circulatory disturbance of the liver, because TGF-ß has an effect of growth inhibition or induction of apoptosis on hepatocytes (Nguyen et al., 2007).

In the peripheral portal tracts of IPH liver, reduction of the immunohistochemical expression of CD34 in the endothelial cells of the peripheral portal vein is frequently observed compared with that of the escorting hepatic artery in the same portal tract. The reduction of CD34 expression is seen in peripheral portal veins of IPH regardless of the presence or absence of luminal narrowing. The reduction of CD34 expression in the portal vein endothelium significantly correlates with the induction of pSmad2 expression, suggesting a causal relationship between them *in vivo*.

Double immunofluorescence staining of CD34 and S100A4 protein shows that portal vein endothelium of IPH occasionally co-expresses CD34 and S100A4 (Fig. 5, arrowhead), suggesting the occurrence of EndMT in the portal vein endothelium. Similarly, co-expression of CD34 and COL1A1 can be observed in the portal vein endothelium of IPH. The portal vein endothelium of normal liver typically lacks such double-positive signals. The portal veins showing the expression of COL1A1 are irregularly distributed in an individual liver independently of the presence or absence of luminal narrowing, suggesting that luminal narrowing of the peripheral portal veins gradually progresses along with collagen deposition.

Despite evidence indicative of the involvement of



CD34/S100A4/DAPI

Fig. 5. Double immunofluorescence staining of CD34 and S100A4 protein of IPH liver. Arrowhead indicates a double-positive signal of CD34 and S100A4 in portal vein endothelium. PV, portal vein.

EndMT, the expression of S100A4 and COL1A1 is limited to only a small fraction of portal endothelial cells of IPH. Similarly, previous studies have shown that the percentage of fibroblast-specific protein-1/CD31-double-positive cells remained at 3% of total cells in a mouse model of cardiac fibrosis, although nuclear staining of pSmad2/3 was present in 30% of endothelial cells (Zeisberg et al., 2007). These results indicate that several, but not all, of the endothelial cells with positive nuclear expression of pSmad2/3 undergo phenotypic changes into myofibroblast-like cells *in vivo*, which may lead to the uneven distribution of stenotic portal tracts in IPH liver.

Co-expression of CD34 and α-SMA is rarely seen in the portal vein endothelium of IPH. These observations are consistent with the results of previous studies regarding EndMT, showing that the occurrence of colocalization of CD31 and α-SMA in the vascular endothelium was a rare event *in vivo*, compared with the frequency of the occurrence of co-localization of CD31 and fibroblast-specific protein-1 (Zeisberg et al., 2007). Therefore, vascular endothelial cells may be able to acquire myofibroblast-like features *in vitro*, but they do not necessarily differentiate into myofibroblasts themselves *in vivo*.

Fibrogenic cytokines

The involvement of fibrogenic cytokines in the disease pathogenesis of IPH has been investigated. It has been shown that connective tissue growth factor (CTGF) is upregulated in the sera and liver tissue of IPH (Tsuneyama et al., 2002; Morikawa et al., 2007). In addition to CTGF, the serum level of TGF-ß1 in IPH patients is significantly higher than the value of healthy controls and patients with chronic viral hepatitis. TGF-ß induces CTGF in various systems. Although the cellular sources of TGF-ß1 have not been addressed, the elevated

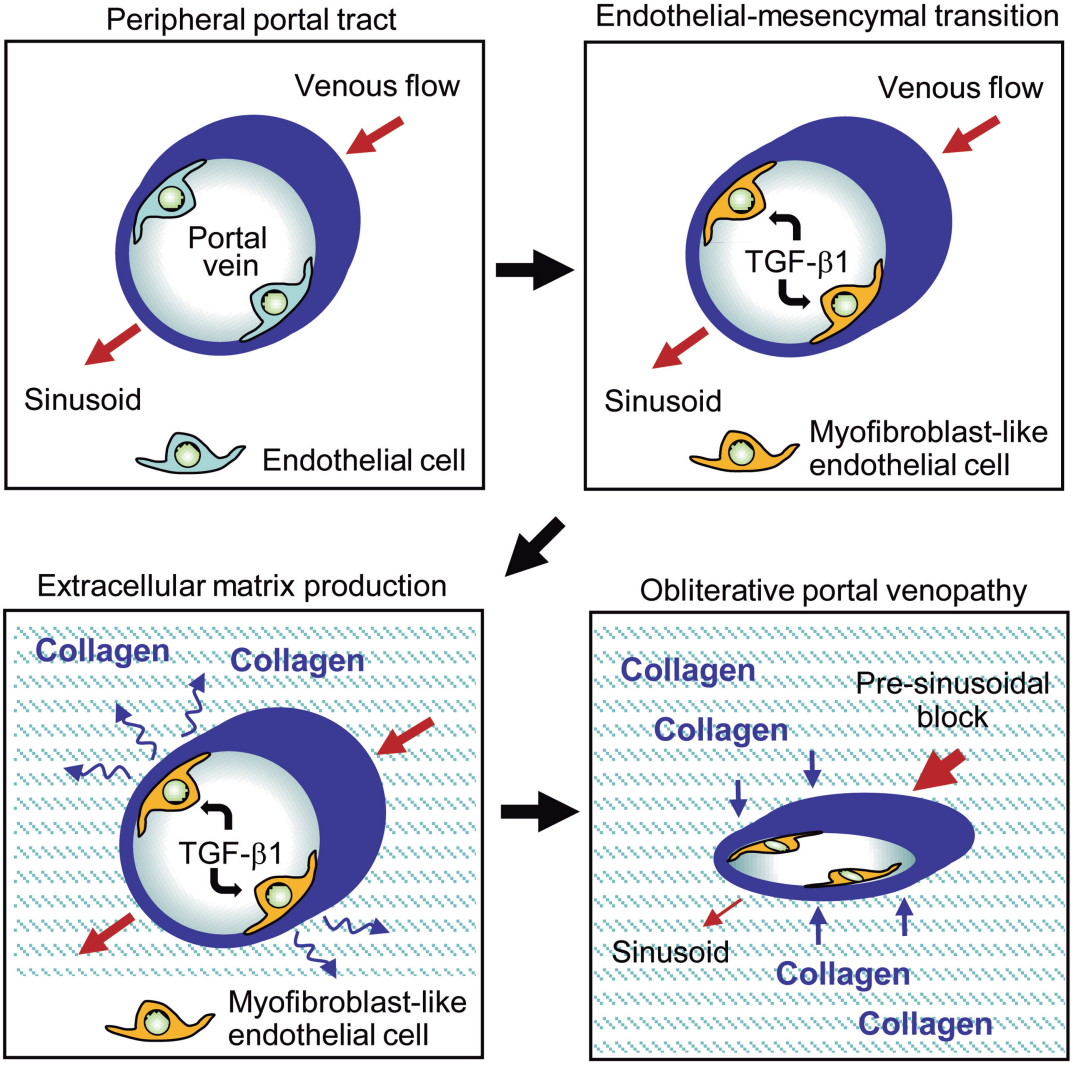


Fig. 6. Proposed pathogenic mechanism of obliterative portal venopathy in IPH. TGF-ß1 induces the process of endothelial-mesenchymal transition (EndMT) in the endothelial cells of peripheral portal vein. Endothelial cells that undergo EndMT acquire the ability to express extracellular matrix molecules such as type I collagen, thereby contributing to obliterative portal venopathy and pre-sinusoidal portal hypertension.

serum TGF-ß1 level may be closely associated with the occurrence of EndMT in IPH.

The serum level of BMP-7, an antagonist of TGF-ß, is not significantly elevated in patients with IPH compared with that of healthy controls, while it is significantly elevated in patients with chronic viral hepatitis. Interestingly, there is a significant inverse correlation between the values of serum TGF-ß1 and BMP-7, suggesting the possibility that TGF-ß1 and BMP-7 have an antagonistic effect on each other's expression.

Taken together, our data suggest that EndMT plays a pivotal role in the dense collagen deposition in the portal tracts of IPHß leading to progression or even initiation of obliterative venopathy. Our proposed pathogenic mechanism of obliterative portal venopathy is illustrated in Fig. 6. From the results of our study, however, it is unclear whether the process of elastic fiber deposition in IPH liver is mediated by EndMT. Elevation of systemic TGF-ß1 level may account for the occurrence of EndMT in the absence of significant local inflammation, and BMP-7 may be a suitable therapeutic candidate for IPH.

Other endothelial dysfunction in IPH

Pathogenic mechanisms of endothelial dysfunction in IPH other than EndMT have been proposed, although the experimental data available are relatively limited because of the small amount of literature.

Immunological disorders

In relation to immunological disorders seen in IPH, the immunohistochemical expression of human leukocyte antigen D-related antigen has been demonstrated on portal microvessels (Terada et al., 1991). Vascular cell adhesion molecule-1 (VCAM-1) is expressed in vascular endothelial cells of the IPH liver, and the serum level of soluble VCAM-1 is elevated in IPH patients (Yamaguchi et al., 1999).

Anti-endothelial cell antibodies (AECA) have been identified as circulating autoantibodies targeting the endothelial cells, and are detectable in a heterogeneous group of autoimmune and inflammatory conditions, including vasculitis (Praprotnik et al., 2001). In SSc, 20-86% of patients exhibit a positive test result for AECA (Mihai and Fervaert, 2010). IPH sera also contain AECA, which may be associated with endothelial cell damage (Sato et al., 2012). Indeed, sera containing AECA have been shown to induce the expression of pathogenic molecules such as VCAM-1 and fibrillin-1, one of the main components of microfibrils that interact with fibulin-5 during elastic fiber assembly, in cultured endothelial cells (Ahmed et al., 2006; Papa et al., 1999). Endothelial damage of the portal system is also postulated in the pathophysiology of IPH developed in patients with human immunodeficiency virus infection who have received drug treatment with didanosine (Schouten et al., 2011).

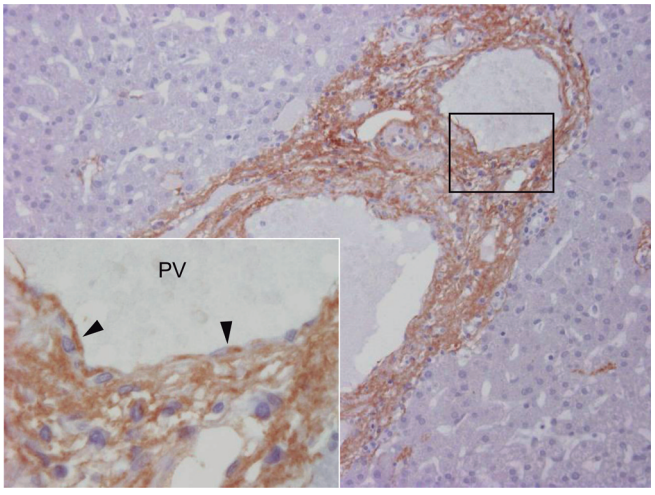


Fig. 7. Immunohistochemical expression of elastin in IPH liver. Arrowheads indicate elastin-positive portal vein endothelial cells. PV, portal vein.

Fibroelastosis

In the peripheral portal tracts of IPH livers, immunohistochemical expression of elastin is observed in the portal vein endothelium, as well as in the portal tracts (Fig. 7, arrowheads). *In vitro*, IPH sera containing AECA induce elastin mRNA expression in HMVEC, and the amounts of elastin mRNA induced in HMVEC correlate significantly with the values of AECA of each serum used for stimulation of HMVEC (Sato et al., 2012). These results suggest a causal correlation between the induction of elastin expression in endothelial cells and the presence of AECA. Thus, AECA may induce elastin expression in endothelial cells, thereby contributing to fibroelastosis in the portal tracts of IPH.

In IPH, fibroelastosis of the major portal vein branches is also a characteristic histological feature. Fibulin-5 is an essential protein that links elastic fibers to cells and regulates fiber assembly and organization (Kielty, 2006). Fibulin-5 is preferably expressed in the vessel walls of the major portal vein branches of IPH, suggesting its role in phlebosclerosis (Sato et al., 2008). While the peripheral portal tracts of IPH totally lack the expression of fibulin-5 in spite of the presence of dense elastic fibers, the mechanism of fibroelastosis may differ between the major portal vein branches and peripheral portal tracts.

Apoptosis

It has been reported that AECA may be pathogenic by inducing endothelial cell apoptosis. Direct and dose-dependent induction of endothelial cell apoptosis by AECA from patients with SSc has been observed

(Bordron et al., 1998). In IPH, sera of patients are capable of inducing apoptosis in HMVEC (Sato et al., 2012). Apoptotic endothelial cells have been shown to secrete CTGF and promote fibrosis (Laplante et al., 2010). These observations suggest that endothelial cell apoptosis is also involved in the disease pathogenesis of IPH.

Splenomegaly

Splenomegaly of IPH is histologically characterized by proliferation of sinus endothelial cells and by irregularly widened interendothelial slits of the sinuses. Sinus lining endothelial cells of the spleen of IPH show diffuse and strong immunohistochemical expression of inducible nitric oxide synthase (iNOS) and endothelial NOS (eNOS) (Sato et al., 2007). Spleen-derived NO may affect the spleen, particularly its sinus lining cells, followed by sinus dilatation. This may in turn lead to massive splenomegaly and increased blood flow to the liver, contributing to sustained portal hypertension in IPH. Thus, the splenomegaly of IPH seems not to be simply passive congestion.

Conclusions

The pathogenic mechanisms of the development of IPH are complex, and seem to be heterogeneous. Endothelial dysfunction of the portal system may be one of the most important causative factors, and in this regard we have recently elucidated a significant role of EndMT in obliterative portal venopathy of IPH. There are limited data available from studies of the mechanism responsible for the development of IPH. To clarify the pathogenic mechanisms and to establish better treatment strategies for IPH, further extensive studies are required.

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Accepted August 31, 2012