Current Research Trends in the Exploration of Therapeutic Targets for Liver Disease

Differential Roles of Angiogenesis in the Induction of Fibrogenesis and the Resolution of Fibrosis in Liver

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Liver fibrosis is a wound healing process that includes inflammation, deposition of extracellular matrix molecules, and pathological neovascularization. Angiogenesis, which is defined by the formation of new blood vessels from pre-existing vessels, is a complex and dynamic process under both physiological and pathological conditions. Although whether angiogenesis can induce or occur in parallel with the progression of hepatic fibrosis has not yet been determined, intrahepatic sinusoidal formation and remodeling are key features of liver fibrosis. Some recent evidence has suggested that experimental inhibition of angiogenesis ameliorates the development of liver fibrosis, while other recent studies indicate that neutralization or genetic ablation of vascular endothelial growth factor (VEGF) in myeloid cells can delay tissue repair and fibrosis resolution in damaged liver. In this review, we briefly summarize the current knowledge about the differential roles of angiogenesis in the induction of fibrogenesis and the resolution of fibrosis in damaged livers. Possible strategies for the prevention and treatment of liver fibrosis are also discussed.

Key words angiogenesis; fibrogenesis; fibrosis resolution; liver; vascular endothelial growth factor

1. INTRODUCTION

Liver fibrosis is a reversible but sustained wound healing process that includes excessive synthesis and subsequent deposition of collagen fibers and extracellular matrix molecules in the hepatic parenchyma.1) Liver cirrhosis, which is the final stage of fibrosis, is characterized by nodular changes and hepatic parenchyma sinusoidal recapillarization. Although the transformation of quiescent hepatic stellate cells (HSCs) into profibrogenic myofibroblasts is believed to be crucial to fibrogenesis, other nonparenchymal cells such as Kupffer cells and endothelial cells (ECs) are also major comodulators of liver fibrosis.2) Angiogenesis is a complex and dynamic process under both physiological and pathological situations.3) Although whether angiogenesis can induce and occur in parallel with the progression of hepatic fibrosis has not clearly been determined, intrahepatic angiogenesis and sinusoidal remodeling are key features of liver fibrosis. Recent studies demonstrated that pathological angiogenesis and sinusoidal remodeling are strongly correlated with progression of chronic liver disease and may act as initiators of liver fibrogenesis.4,5) The pathological vascularization and development of abnormal angio-architecture in the liver are associated with progressive fibrogenesis, which in turn leads to liver cirrhosis and hepatic cancer.6) Therefore, links between fibrogenesis and angiogenesis may provide novel clues to the mechanism of and development of therapeutic strategies against liver fibrosis. Accumulating experimental evidence suggests that the inhibition of angiogenesis ameliorates the development of liver fibrosis. In particular, both vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are suggested to play pivotal roles in angiogenesis and vascular remodeling by the regulation of EC survival and proliferation. In vivo inhibition of VEGF provides convincing evidence for its contribution to fibrogenesis and portal hypertension, and VEGF overexpression accelerates fibrosis by increasing hepatic collagen deposition.7) Therefore, strategies targeting VEGF inhibition are already being used to treat human diseases such as cancers and are being considered for the treatment of liver fibrosis.8) In contrast, a more recent study suggests that the neutralization or genetic ablation of VEGF in myeloid cells can promote tissue repair and resolve fibrosis in damaged livers.9) Depending on the stage of differentiation, liver sinusoidal endothelial cells (LSECs) play dual roles in hepatic fibrogenesis and fibrosis resolution.

2. LIVER FIBROSIS

Liver fibrosis is a reversible wound-healing response initiated by cellular communication that reflects a balance between liver repair and scar formation. Although acute liver injury often initiates fibrogenesis, such changes in the liver are transient and easily reversible. In chronic liver disease, collagen and various extracellular matrix (ECM) molecules accumulate in liver parenchyma, followed by the disruption of normal architecture and progression to fibrosis and cirrhosis. HSCs are primary effector cells, orchestrating the deposition of ECM during liver fibrosis. HSCs are resident liver immune cells positioned in the space of Disse between sinusoidal endothelial cells and hepatocytes.10)

Following liver injury, HSCs undergo activation and transformation from quiescent vitamin A-storing cells to prolifera-
transformation of myofibroblast-like cells. As described by Friedman, HSC activation requires initiation and perpetuation steps, followed by a resolution phase if the injured liver is recovered. Initiation refers to early events in which HSCs respond to a variety of cytokines, growth factors, and other stimuli. Initial stimuli include paracrine signals such as reactive oxygen species or damage-associated molecular patterns (DAMPs) from damaged hepatocytes and signals from activated Kupffer cells and ECs. Type 1 collagen, α-smooth muscle actin (α-SMA), transforming growth factor β (TGF-β), matrix metalloproteinase (MMP)-2, and tissue inhibitors of metalloproteinase (TIMP) are considered potential target genes of transcription factors in HSCs. Perpetuation maintains an activated HSC phenotype and generates fibrosis. Signals such as cytokines and growth factors generate scar tissue through sustained activation phases such as cellular proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis, retinoid loss, and inflammatory signaling. During this step, proinflammatory and profibrogenic cytokines are released in autocrine and paracrine pathways.

The resolution of liver fibrosis is a valuable therapeutic step in a process including degradation of matrix and low-density basement membrane, and clearance of HSCs by apoptosis. In addition to these changes, HSCs activate immune responses by secreting inflammatory cytokines and chemokines and interacting with neighbor cells such as Kupffer cells and ECs. HSCs also contribute to the regulation of oxidative stress and vascular stability, maturation of ECs, and remodeling.

3. INITIATION AND REGULATION OF ANGIOGENESIS

Hypoxic conditions are thought to be a primary trigger for angiogenesis in ischemic tissues, in which hypoxia inducible factor (HIF) expression is induced in ECs and HSCs. HIF-1α then activates the transcription of genes such as VEGF, which are directly or indirectly involved in angiogenesis. VEGF is a major regulator of vascular formation and remodeling through effects on EC activation and proliferation. VEGF is mediated via two tyrosine kinase receptors, VEGF receptor 1 (VEGFR-1, Flt-1) and VEGFR-2 (KDR, Flk-1), which are mainly expressed on EC. The roles of VEGF receptors in vascular development have been extensively studied. VEGFR-1 knockout mice show overgrowth of immature vessels and blocked vessel development, while VEGFR-2 knockout mice show deficiencies in blood vessel formation. VEGF interacts with endothelial precursor cells such as angioblasts and differentiating or mature ECs. Stimulated ECs secrete several plasminogen activators and matrix metalloproteases, and these molecules degrade the ECM and vascular basement membranes of parental vessels, which may help generated ECs invade the surrounding matrix. The immature ECs proliferate and migrate to new sites where the hypoxia and hyperplasia of ECs are accelerated to form solid endothelial tubes. Simultaneously, DNA synthesis and division of ECs occur due to the actions of mitogenic agents, and ECs and pericytes synthesize basement membrane and form lumina. Pericytes penetrate into the segmental centers of lumina and compose the structures of new capillaries. Finally, blood vessel maturation and stabilization occur and ECs secrete growth factors such as PDGF, which attract supporting cells to stabilize the new vessel. New capillary ECs are differentiated into pericytes, smooth muscle cells, and other tissues. These complex but highly ordered angiogenesis steps are also directly or indirectly affected by several other factors, such as angiogenin, leptin, and TGF-β.

4. ANGIOGENESIS IN LIVER FIBROSIS

Angiogenesis is a critical step in a number of pathological conditions associated with hepatocyte damage, wound healing, and remodeling in the liver. Recent experimental and clinical studies clearly demonstrate that liver fibrogenesis is accompanied by the formation of new vessels and the establishment of abnormal angioarchitecture of the liver, which are closely associated with the development and progression of liver fibrosis. Cumulative evidence indicates that liver fibrosis involves intrahepatic vascular remodeling with capillarization, which is characterized by lack of LSEC fenestration and formation of an organized basement membrane.

In fibrotic liver, venous pressure is increased by deposition of ECM. When portal venous resistance increases beyond normal compensatory ability, oxygen delivery is reduced. Hypoxia acts as an aggravating factor of cell damage and inflammation as well as a most potent stimulant for angiogenesis, with the transcription of hypoxia-sensitive pro-angiogenic genes modulated through HIFs. Hypoxia is a major factor implicated in the induction of VEGF through a pathway that involves the transcription factor HIF-1α. VEGF binds to receptors located on the surfaces of ECs in order to induce the formation of new blood vessels. Recent studies have shown that VEGF is upregulated in hepatocytes in fibrotic livers, and that VEGF expression colocalizes with hypoxic areas. VEGF is also upregulated in hypoxic hepatocytes, and inhibition of HIF-1α signaling completely prevents up-regulation of VEGF in such cells.

Although hepatocytes are the main cells producing VEGF in the liver, several studies have demonstrated that hypoxia also stimulates VEGF expression in HSCs, and others have observed an important autocrine VEGF signaling loop within ECs themselves. HSCs induce fibrosis via collagen and proinflammatory cytokine production but are increasingly recognized for their roles in angiogenesis and vascular remodeling. Although LSECs and endothelial progenitor cells (EPCs) are the main cell types that participate in angiogenesis, pericytes also play an important role in vascular stabilization, maturation, and remodeling. HSCs are liver-specific pericytes that are found around the vasculature of the hepatic sinusoid and in the space of Disse between hepatocytes and LSECs. HSCs can release pro-angiogenic molecules including VEGF. During angiogenesis, LSECs synthesize and secrete PDGF, which acts on PDGF receptors expressed on the surfaces of HSCs, in response to the secreted VEGF. The level of PDGF production is a major determinant of HSC recruitment to newly formed blood vessels through specific chemotactic gradients that are generated and regulated by ECs. In fibrotic livers, recruited HSCs can modulate the maturation of new vessels and provide stability and durability to newly formed vessels that cannot be achieved by LSECs alone in the absence of pericytes such as HSCs. Inflammation is a pathophysiological response to stimuli, and initiates
the wound healing process after tissue damage. Angiogenesis may be more important for perpetuating rather than initiating inflammatory responses. New blood vessels can maintain chronic inflammation by transporting inflammatory cells to injured sites, and by supplying nutrients and oxygen to regenerating inflamed tissues.65)

Large numbers of neovessels and massive inflammatory responses are observed during the development of liver fibrosis.29) Multiple lines of evidence suggest that many pro-inflammatory molecules, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and nitric oxide (NO), directly induce angiogenesis.30,31) Kupffer cells act not only as phagocytic cells, but also as obligatory regulators of inflammation and fibrosis by producing various cytokines, chemokines, and reactive oxygen and nitrogen species.32,33) The chemokine-dependent accumulation of monocyte-derived macrophages has been identified as an important mechanism for perpetuating hepatic inflammation and promoting fibrogenesis in experimental mouse models and in human liver diseases.34) CCL2-dependent infiltrating macrophages accumulate in large numbers in newly formed vessels of fibrotic liver and produce pro-angiogenic molecules, including VEGF and MMP-9.35) Activated HSCs produce a number of chemokines regulated by pro-inflammatory cytokines and recruit inflammatory cells.36) HSCs can also express cell adhesion molecules such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 to mediate inflammatory cell infiltration in injured livers.37)

Most newly formed vessels are immature and vulnerable, so it is difficult to alleviate the ischemic state of a fibrotic liver. Under conditions of persistent hypoxia, the continuous production of pro-inflammatory and angiogenic molecules eventually stimulates ECM deposition and liver fibrosis. Hence, hypoxia, pathologic angiogenesis, excessive inflammation and fibrosis may act synergistically and thereby exacerbate the severity of liver fibrosis.38)

5. ANGIOGENESIS-RELATED THERAPY FOR THE TREATMENT OF LIVER FIBROSIS

The close relationship between the progression of liver fibrosis and angiogenesis suggests that anti-angiogenic therapy may be a useful tool for preventing and managing fibrogenic progression. Pioneering studies employing drugs such as the semi-synthetic analogue of fumagillin TNP-470, which is an angiogenesis inhibitor, indicate that TNP-470 inhibits HSC proliferation by blocking the cell-cycle transition from G1 to S, HSC activation, and the deposition of ECM in liver. Hence, TNP-470 impedes the progression of hepatic fibrosis, probably by being coupled with its anti-angiogenic effect.39)

Since VEGF is one of the most potent pro-angiogenic factors, most anti-angiogenic therapies have focused on blocking the VEGF signal pathway. Another effective anti-angiogenic approach is the administration of neutralizing antibodies targeting VEGFR1 and VEGFR2. Although combination treatment with both antibodies is slightly more effective than treatment with either alone, anti-VEGFR2 antibody is more effective than anti-VEGFR1 antibody when used alone, suggesting that VEGF interaction with VEGFR2 rather than VEGFR1 contributes to angiogenesis during liver fibrosis.36) Bevacizumab can attenuate VEGF-induced angiogenesis and vascular malformation.39) In rat liver fibrosis models, treatment with bevacizumab alleviates liver fibrosis by neutralizing VEGF produced by hepatocytes and blocking its effects for the activation of HSCs.40) Recent anti-angiogenic approaches include use of the multiple receptor tyrosine kinase inhibitor Sorafenib to target the Raf/extracellular signal-regulated kinase (ERK) and PDGFR-β signaling cascade, as well as the VEGFR signaling pathway. Sorafenib treatment attenuates liver fibrosis and is associated with significant decreases in intrahepatic fibrogenesis and collagen deposition. Moreover, sorafenib reduces HSC proliferation and induces apoptosis of HSC.41) Several experimental studies have shown that ECM degradation and spontaneous resolution of liver fibrosis are accompanied by apoptosis of activated HSCs.42) Treatment with sunitinib, a more recently-developed anti-angiogenic drug designed to inhibit tyrosine kinase receptors, also results in significant decreases of portal pressure, inflammation, HSC activation and ECM deposition, in addition to angiogenesis.43)

Although these previous experimental studies have focused on anti-angiogenic therapy in order to prevent the progression of fibrogenesis, experimental angiogenic therapy for fibrosis resolution has received increasing attention in recent years. Recent pre-clinical and clinical studies have indicated that the transplantation of bone marrow cells (BMCs) and EPCs can ameliorate abnormal angiogenesis and hepatic fibrosis. Regenerative therapy targeting liver fibrosis using BMCs reduces the severity of liver fibrosis by increasing the degradation of ECM via enhanced MMP expression, ultimately improving morbidity and mortality.44) EPC transplantation was shown to effectively promote the remodeling of tissues damaged by liver fibrosis in the dimethyl nitrosamine (DMN) rat liver fibrosis model.45,46) In a recent experimental study, VEGF played a dual and opposing role in liver fibrogenesis and fibrolysis through critical effects of VEGF on LSEC phenotype, sinusoidal permeability, monocyte infiltration, and scar-associated macrophage (SAM) function. Mechanistically, a VEGF-driven C-X-C motif chemokine 9 (CXCL9)-MMP13 axis was identified for mediating fibrosis resolution.6) VEGF also plays an important role in determining the phenotype of LSEC. LSEC with normal fenestration and function prevents HSC activation and promotes reversion to the quiescent state of HSC, but capillarized LSEC with denfenestration and malfunction does not.47) LSEC fenestration is maintained by VEGF secreted by hepatocytes or HSCs and VEGF downstream signaling produces nitric oxide (NO), which acts through the soluble guanylate cyclase (sGC)/cyclic guanosine monophosphate (cGMP)/protein kinase G pathway.48–50) Restoration of LSEC through reversal of capillarization by pharmacological therapy to activate the VEGF-NO-related signaling pathway accelerates HSC quiescence and regression of fibrosis.47)

In the fibrotic liver, myeloid cells and particularly macrophages constantly infiltrate into fibrotic scar areas. Although SAMs play a pro-fibrogenic role during the development of fibrosis, the MMP expression of macrophages contributes to the resolution of fibrosis.51) In line with this idea, the resolution of liver fibrosis is associated with increased expression of MMP-2 and -14 as well as decreased expression of TIMP-1 and -2 in sinusoidal endothelium. Myeloid cell-derived VEGF also drives a pro-resolution phenotype in LSECs and induces revascularization in fibrotic scar tissue. Genetic inactivation of VEGF in scar-infiltrating macrophages prevents the angio-
6. CONCLUDING REMARKS

Most relevant studies support the hypothesis that pathologic angiogenesis plays a critical role in liver fibrosis accompanied by chronic injury, persistent inflammation and progressive fibrogenesis. Therefore, developing effective therapeutic strategies to control the formation of new capillary ECs is essential for the treatment of liver fibrosis and its complications. However, since such experimental approaches are limited to mostly pre-clinical trials, the side effects of long-term...
administration of anti-angiogenic agents should be taken into consider­ation when using anti-angiogenic therapies in patients with liver fibrosis. Recent studies have highlighted the impor­tance of experimental angiogenesis-inducing therapy to treat liver fibrosis, and VEGF has been observed to play a dual and opposing role in liver fibrosis and fibrosis resolution (Fig. 1). Furthermore, physiological angiogenesis plays a critical role in the normal wound healing response. Therefore, appropriate control strategies that consider the degrees and stages of neo­vascularization and liver fibrosis must be developed to avoid excessive inhibition of normal angiogenic responses.

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