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Vascular factors, angiogenesis and biliary tract disease

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Abstract

Purpose of review—Recent studies have brought to light that angiogenesis and the expression of proangiogenic factors such as vascular endothelial growth factors (VEGFs) participate in the pathogenesis of biliary tract diseases. This review summarizes recent progress that has been accomplished in the field, which expands our understanding of the relationship between vascular growth and the biliary tract, particularly the molecular mechanisms that underlie the pathogenesis of biliary tract diseases.

Recent findings—Angiogenesis and the expression of vascular factors play a key role in the pathogenesis of primary biliary cirrhosis, cholangiocarcinoma, liver cysts, and in the progression of biliary fibrosis in animal models. Inhibition of angiogenesis limits fibrosis in animal models, whereas the bile acid, taurocholate, has protective effects in animal models of bile duct and peribiliary vascular plexus damage.

Summary—A widening body of information indicates that the expression of proangiogenic factors such as VEGFs and angiogenesis play an important role in a variety of biliary tract diseases. Further characterization of the link between angiogenesis and vascular growth factor expression will help in elucidating the mechanisms regulating the pathogenesis of biliary tract diseases and in devising new treatment approaches for these devastating diseases.

Keywords

angiogenesis; biliary epithelial cells; cholangiocytes; cholestatic liver disease; vascular endothelial growth factor

Introduction

The formation of new vascular structures, angiogenesis, occurs during disease pathogenesis in multiple organ systems. The role of vascular growth factors and angiogenesis in biliary tract diseases [such as primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC) and cholangiocarcinoma] has recently become the topic of interest from the standpoint of disease pathogenesis [1,2]. The function of the intrahepatic biliary tree is very closely linked to its vascular supply, the peribiliary vascular plexus (PBP) [3]. Alterations of the intrahepatic biliary tree during cholestasis are associated with changes in the architecture of the PBP [4]. In order to support the increased nutritional and functional demands from proliferating bile ducts during

bile duct ligation (BDL), the PBP undergoes marked proliferation [4]. Interestingly, the proliferation of the PBP occurs only after hyperplasia of the intrahepatic biliary epithelium during extrahepatic cholestasis [4]. In addition to the close link between the proliferating cholangiocytes and the PBP, proliferating biliary epithelial cells (i.e. cholangiocytes) display neuroendocrine phenotypes, and as such proliferating cholangiocytes secrete and respond to a number of hormones, neuropeptides and neurotransmitters [2]. During the course of cholestasis, cholangiocytes undergo a neuroendocrine transdifferentiation and their biology is thereby regulated by a number of factors, such as vascular endothelial growth factor (VEGF) [2]. VEGF-A (the central mediator of angiogenesis) [5•] and VEGF-C (a factor regulating lymphogenesis) [6•] are secreted by cholangiocytes [7,8]. These factors play a key role in the regulation of biliary proliferation during cholestasis in an autocrine mechanism through VEGF receptors expressed by cholangiocytes (VEGFR-2 and VEGFR-3) [7,8]. For example, during hepatic artery ligation (HAL)-induced ischemia of the biliary system, the PBP disappears and cholangiocytes are characterized by increased apoptosis, decreased proliferation, and decreased VEGF-A secretion [7]. Administration of VEGF-A prevented the ischemic injury induced by HAL by restoring cholangiocyte proliferation and maintaining the integrity of the PBP (Fig. 1) [7]. These seminal studies demonstrated the clear link between biliary proliferation, VEGF and the PBP, and established the interest in the potential role for vascular factors and angiogenesis during the pathogenesis of cholangiopathies. This article reviews the recent advances in the understanding of the roles of vascular factors and angiogenesis on regulating cholangiocyte proliferation and during the progression of biliary tract diseases.

Cholestatic liver diseases and biliary fibrosis

Cholangiocytes are the target cells in a number of cholangiopathies including PBC and PSC, diseases associated with dysregulation of the balance between cholangiocyte proliferation/apoptosis [9]. Immune-mediated cholestatic liver diseases such as PBC and PSC are characterized by biliary damage and chronic inflammation [9]. During chronic inflammatory liver diseases, new vessel formation plays an important role in tissue remodeling as well as the delivery of nutrients and oxygen to areas of hypoxia induced by chronic inflammation [10]. In an immunohistochemical study, Medina *et al.* [11] demonstrated that intrahepatic angiogenesis occurs in PBC tissue samples, which involved neovessel formation and enhanced expression of VEGF-A, angiopoietins 1 and 2 (Ang 1 and Ang 2), the angiopoietin receptor (Tie-2), and endoglin in the inflamed portal areas. In PBC as well as PSC, the newly created vessels are thought to provide a potential pathway for the recruitment of inflammatory infiltrate, such as T lymphocytes [11]. A recent study has provided additional evidence that angiogenesis plays a role in the protection of cholangiocytes from damage in an experimental model of cholestasis [12•]. In an animal model of cholestasis and biliary damage induced by caffeic acid, the feeding of the protective bile acid taurocholate prevented bile duct damage, which was associated with increased cholangiocyte VEGF-A, VEGF-C, VEGFR-2 and VEGFR-3 expression [12•]. Although this study did not evaluate alterations in the PBP, the findings suggest that bile acids may play a role in the regulation of VEGF and VEGFR expression and have potential to regulate PBP growth during cholestasis. Recent evidence from our group indicates that taurocholate can also protect cholangiocytes and the PBP from HAL-induced damage [13] (Glaser *et al.*, unpublished data). The protective effect of taurocholate was due to elevations in VEGF-A expression in the circulation and in cholangiocytes [13] (Glaser *et al.*, unpublished data). In-vitro studies indicate that taurocholate stimulates the expression and secretion of VEGF-A [13] (Glaser *et al.*, unpublished data). The mechanisms of the protective effect of taurocholate during bile duct damage are illustrated in Fig. 2. The actual intracellular signaling mechanisms by which bile acids can regulate the expression of VEGF and VEGFR receptors remain undefined. However, the bile acids may be useful for the modulation of VEGF expression.

Aberrant angiogenesis is implicated in the progression of hepatic fibrosis and is considered to be a major determinant in the irreversibility of fibrosis [14]. Hepatic stellate cells (HSCs) are a major contributor to hepatic fibrosis [14]. In cirrhotic livers, myofibroblastic HSC (MF-HSC) and cholangiocytes both produce Hedgehog ligands [15]. Activation of the Hedgehog signaling pathway in cholangiocytes by Hedgehog ligands secreted by MF-HSC stimulates the production of Cxcl16, a chemokine that recruits natural killer (NK) T cells to portal tracts [16•].

Interestingly, both platelet-derived growth factor-treated MF-HSC and cholangiocytes release exosome-enriched microparticles containing Hedgehog ligands [17••]. BDL increases the release of Hedgehog-containing microparticles in bile and plasma, which when incubated with hepatic sinusoidal endothelial cells (SECs) stimulate gene expression changes that are known to occur as SECs undergo capillarization [17••]. These studies implicate Hedgehog signaling in fibrosis, inflammation and angiogenesis during chronic liver disease [15,16•,17••]. In addition to the involvement of Hedgehog in promoting angiogenesis, Taura *et al.* [18••] demonstrated that activated HSCs also secrete the proangiogenic factor, Ang 1. Adenoviral expression of soluble Tie2 prevented both angiogenesis and liver fibrosis induced by carbon tetrachloride (CCl₄) and BDL [18••]. In addition, nonspecific inhibition of angiogenesis with sunitinib has been shown to reduce fibrosis [19]. More recently, sorafenib, a potent inhibitor of the proangiogenic VEGFR-2 receptor, reduced portal hypertension and improved liver damage and intrahepatic fibrosis in animals with cirrhosis induced by BDL [20•]. Collectively, these studies indicate that antiangiogenic therapies might be beneficial for chronic liver diseases. However, a recent study suggests that caution is advised for the utilization of inhibitors of angiogenesis in patients with hepatic fibrosis [21••]. In this study, a specific inhibitor of $\alpha\beta 3$ integrin (Cilengitide) was administered to rats with BDL [21••]. Integrin complex $\alpha\beta 3$ promotes angiogenesis by mediating migration and proliferation of endothelial cells, but also drives the activation of HSC and is highly expressed by proliferating bile ducts during fibrosis [22•,23]. Cilengitide inhibited angiogenesis, but worsened biliary fibrosis *in vivo*, despite antifibrogenic effects on HSCs *in vitro* [21••]. Another $\alpha\beta 3$ integrin antagonist, EMD527040, was also shown to inhibit cholangiocyte proliferation and reduce biliary fibrosis [22•]. These findings indicate the potential for the inhibition of angiogenesis as a potential therapy. However, future studies are needed to determine feasibility in humans.

Cholangiocarcinoma

Cholangiocarcinoma results from the malignant transformation of cholangiocytes [24]. The pathogenesis of cholangiocarcinoma is linked to chronic biliary inflammation, which occurs in cholestatic liver diseases such as PSC [24]. Cholangiocarcinoma cell lines and human tumor samples have been shown to express VEGF-A and VEGFRs [25,26]. The role of VEGFs in cholangiocarcinoma proliferation in both in-vitro and in-vivo models has been addressed in several recent studies. Estrogens have been shown to cooperate with insulin-like growth factor (IGF1) and its receptor (IGF1-R) to simulate the growth of cholangiocarcinoma [25]. Estrogens also stimulate the expression and secretion of VEGF-A, VEGF-C and VEGFRs in cholangiocarcinoma cell lines potentially altering cholangiocarcinoma proliferation and tumor neoangiogenesis [27••]. Other studies have shown that factors that inhibit cholangiocarcinoma proliferation also inhibit VEGF expression. Endothelin 1 (ET-1) inhibited the proliferation of cholangiocarcinoma xenografts in nude mice, which was associated with a down-regulation of VEGF-A and VEGF-C expression [28•]. (R)-(alpha)-(-)-methylhistamine dihydrobromide (RAMH), an H3 histamine receptor antagonist, has also been shown to decrease the proliferation of cholangiocarcinoma cells *in vitro* via activation of the H3 histamine receptor through the stimulation of protein kinase C- α (PKC α)-dependent down-regulation of ERK1/2 phosphorylation [29•]. *In vivo*, RAMH decreased tumor growth and the expression of VEGF-A and VEGF-C and its receptors [29•]. In the BDL model of extrahepatic cholestasis,

RAMH also inhibits biliary growth of BDL rats by down-regulation of the cAMP-dependent PKA/ERK1/2/ELK-1 pathway [30]. These studies indicate that there is a causal link between cholangiocarcinoma proliferation and the expression of VEGF-A and VEGF-C and its receptors. In tumor samples, a large number of intrahepatic and extrahepatic cholangiocarcinomas overexpress epidermal growth factor receptor (EGFR) and VEGF [31•]. EGFR expression is associated with tumor progression and VEGF expression may be involved in metastasis in cholangiocarcinoma [31•]. In fact, inhibiting both VEGFR and EGFR signaling with vandetanib (ZD6474) appears to be promising therapy for cholangiocarcinoma that lack KRAS mutations and/or have EGFR amplification [32•]. Cholangiocarcinoma cell lines possessing KRAS mutations were refractory to vandetanib [32•].

Other biliary tract diseases

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by an abnormal development of the renal tubules and by a liver manifestation that is characterized by the presence of biliary cysts and aberrant portal vasculature [33••]. Cholangiocytes that line the liver cysts in ADPKD have phenotypic and functional similarities with reactive ductules [1]. VEGF-A, VEGFR-1 and VEGFR-2 expression is highly up-regulated in the cystic epithelium in liver cysts from patients with ADPKD [34]. Cholangiocytes isolated from the ADPKD liver cysts secreted VEGF and exogenous VEGF-A stimulated the proliferation of cholangiocytes [34]. Spirli *et al.* [35••] have recently shown that VEGF-A promotes the growth of liver cysts in polycystic kidney disease 2 (Pkd2) gene knockout mice through the activation of PKA-ERK1/2-dependent signaling mechanisms. They also demonstrated that activation of PKA-dependent ERK1/2 signaling by VEGF-A stimulated HIF-1 α -dependent VEGF-A secretion [35••]. Another recent study has also shown that factors secreted by liver cyst epithelia can activate VEGF signaling pathways and induce endothelial cell proliferation and differentiation [33••]. Taken together these findings suggest that inhibition of VEGF signaling might be used to treat patients with polycystic liver disease. A working model of the signaling mechanisms is illustrated in Fig. 3. VEGF has also been implicated in the pathogenesis of biliary atresia. Biliary atresia is a chronic inflammatory disease of the bile ducts resulting in biliary cirrhosis and the most perplexing cause of neonatal cholestasis [36]. Genetic variation in the VEGF gene in particular the VEGF-A +983 C/T polymorphism and the VEGF-C allele are associated with biliary atresia and may confer increased susceptibility to the disease [37•].

Conclusion

Angiogenesis and the expression of vascular factors (such as VEGF) are intimately involved in the pathogenesis of biliary tract diseases. The studies reviewed in this article raise the possibility for utilizing the inhibition of angiogenesis and VEGF signaling for the treatment of various biliary tract diseases ranging from biliary fibrosis and cholangiocarcinoma to liver cysts in patients with polycystic liver disease. Future studies will be needed to determine if antiangiogenic treatments are well tolerated and effective in patients with these particular cholangiopathies. Studies are also needed to determine the role that VEGF plays in the neovascularization of cholangiocarcinoma tumors. In the case of biliary damage, administration of VEGF receptor agonists might be used to protect the bile ducts against damage and promote the maintenance of the PBP during hepatic artery ischemia. In addition, studies are needed to determine the role of bile acids in the regulation of VEGF and VEGFR expression during cholestasis. Other studies will need to address the role that VEGF plays in aberrant angiogenesis during biliary fibrosis and how cholangiocytes interact with other liver cell types such as HSC and vascular endothelial cells. Manipulation of the signaling mechanisms that regulate angiogenesis may thus represent a new approach to treating cholangiopathies.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 292).

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Figure 1.

Scanning electron microscopy of vascular corrosion cast from rats (immediately after BDL +HAL) treated by intraperitoneally implanted Alzet osmotic minipumps with 0.2% BSA or r-VEGF-A with 0.2% BSA for 1 week. Observe in BDL +HAL rats the presence of a sinusoidal network (s) and portal vein (p) and the absence of the peribiliary plexus (PBP) (original magnification $\times 50$); differently, in BDL +HAL +r-VEGF-A rats the PBP that runs together with the portal tract was observed (original magnification $\times 100$) Reproduced with permission from [7].

Figure 2. Working model of the protective effects of taurocholate during bile duct damage

In animal models, when proliferating cholangiocytes are damaged by HAL or chemical insults such as caffeic acid, feeding of the bile acid taurocholate prevents cholangiocyte damage via the up-regulation and/or maintenance of VEGF secretion from cholangiocytes. In the case of HAL, the feeding of taurocholate also helps to maintain the integrity of the PBP.

Figure 3. Regulation of polycystic liver cyst growth and angiogenesis by VEGF-A

Biliary epithelial cells from liver cysts secrete VEGF-A, which stimulates proliferation of the cysts via the activation of PKA/ERK1/2 signaling and stimulation of increased VEGF-A secretion. VEGF-A can also stimulate the proliferation and differentiation of endothelial cell proliferation presumably resulting in angiogenesis. Blockade of VEGF-A signaling by VEGFR antagonist represents a potential therapy for ADPKD liver cysts.