

Mechanisms of angiogenesis in chronic inflammatory liver disease

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Abstract

Intrahepatic hypoxia may occur during the inflammatory and fibrotic processes that characterize several chronic liver diseases of viral and autoimmune origin. As a consequence, new vascular structures are formed to provide oxygen and nutrients. Angiogenesis involves a tightly regulated network of cellular and molecular mechanisms that result in the formation of functional vessels. Of particular importance are growth factors and molecules involved in matrix remodeling and cell migration, as well as vessel maturation-related factors. In recent years a number of studies have investigated the expression and function of many pro- and antiangiogenic molecules in chronic liver diseases and liver regeneration. This review examines the potential pathogenic role of angiogenesis in the context of viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis and hepatocellular carcinoma.

Key words: Chronic liver disease, adhesion molecules, angiogenesis, vascular endothelial growth factor, angiopoietins.

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Abbreviations

E-selectin, endothelial selectin; ECM, extracellular matrix; EC, endothelial cells; NO, nitric oxide; VEGF, vascular endothelial growth factor; aFGF and bFGF, acidic and basic fibroblast growth factors; HGF, hepatocyte growth factor; KDR, kinase insert domain receptor; Flt-1, fms-like tyrosine kinase receptor; CHC, chronic hepatitis C; HCV, hepatitis C virus; Ang, angiopoietin; ALT, alanine aminotransferase; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; AIH, autoimmune hepatitis; HCC, hepatocellular carcinoma.

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Introduction

Angiogenesis, the formation of new vascular structures from preexisting ones, occurs in several organs during multiple pathophysiological situations.¹ It has become one of the most thoroughly investigated pathophysiological phenomena in the last few years because of the key roles it plays in disease pathogenesis and its potential as a therapeutic target. Although mostly studied in relation to cancer, angiogenesis is also known to occur in pathologies characterized by chronic inflammation. Recent work from our group and others has demonstrated that chronic diseases of the liver do not represent an exception to this rule.

In 1995, our group² studied by immunohistochemistry the intrahepatic expression pattern of vascular adhesion molecules (CD31, E-selectin, VCAM-1, cadherin 5 and endoglin) in viral chronic hepatitis. A novel and striking finding in this study was the detection of CD31+ and cadherin 5+ endothelial cells with microvessel morphology in the inflamed portal tracts from patients with viral chronic hepatitis. Furthermore, the expression pattern of both CD31 and cadherin 5 molecules on endothelial cells was always similar in enlarged portal tracts of these patients, acquiring a characteristic form of capillary tube formation. The assumption for an endothelial cell specificity was further reinforced, because cadherin 5 is a selective endothelial cell marker. It was also interesting to note that the presence of these portal microvessels was more important in those cases where portal tracts were more inflamed. By contrast, a weak immunoreactivity for CD31, cadherin 5, and endoglin was only found on sinusoidal endothelial cells and on portal vascular endothelium in normal liver tissue. This work gave important information about the potential pathogenetic role of angiogenesis in the context of liver disease.

Mechanisms of angiogenesis

Hypoxia and inflammation are the main stimulus for angiogenesis. Hypoxia promotes the angiogenesis by signalling through hypoxia inducible transcription factors.^{1,3} During inflammation, vascular permeability is increased and monocytes, macrophages, platelets, mast cells, and other leukocytes (able to produce angiogenic

cytokines and growth factors) are recruited under the attraction of chemokines^{4,5} (Figure 1). The formation of new functional vessels involves different processes whose molecular effectors must be precisely regulated.^{6,7} Medina et al¹ reviewed the evidence for angiogenesis in chronic inflammatory liver diseases and analyzed the cellular and molecular mechanisms involved.

Endothelial budding is facilitated by vasodilation, loosening of interendothelial contacts, and leakiness of pre-existing vessels, which allows extravasation of plasma proteins that, together with extracellular matrix components (ECM), lay down a provisional scaffold for migrating endothelial cells (EC). Nitric oxide (NO), whose angiogenic properties have been characterized,⁸ is the main factor responsible for vasodilation, whereas vascular endothelial growth factor (VEGF) increases vascular permeability. Next, the basement membrane (mainly collagen IV and laminin) and the ECM (collagen I and elastin) must be degraded to allow subsequent EC migration and proliferation. This is performed by specialized proteinases, including plasminogen activator. ECM proteolysis leads to the exposure of cryptic epitopes and release of ECM-embedded factors that promote EC migration and proliferation.^{9,10}

EC proliferate in response to growth factors secreted by EC or surrounding cells (including hepatic stellate cells, leukocytes, hepatocytes and Kupffer cells). The most thoroughly characterized is VEGF, a multifunctional protein that binds to 2 tyrosine kinase receptors: kinase insert domain receptor (KDR) and fms-like tyrosine kinase receptor (Flt-1).¹¹ The VEGF promoter contains hypoxia-inducible factors-responsive elements. VEGF

plays a crucial role in virtually all pathological situations in which angiogenesis occurs, and the therapeutic use of strategies aimed at blocking its mode of action is currently under evaluation.¹² In addition, EC proliferation may be stimulated by other growth factors, such as acidic and basic fibroblast growth factors (aFGF and bFGF); hepatocyte growth factor (HGF); and transforming growth factor (TGF).^{7,13}

EC proliferate in an ordered manner that leads to the formation of a lumen.^{14,15} A structured 3-dimensional network of vessels of uniform size is then organized by carefully regulated mechanisms involving signaling pathways that determine branching, formation of basement membrane and ECM components, and cell migration and differentiation. For nascent vessels to mature, pericytes must be recruited, and a new basement membrane and ECM must be generated to provide structural stabilization.¹⁵ Physical forces and multiple molecules contribute to these processes.

Besides induction of EC proliferation, effective angiogenesis also requires stabilization of the nascent vessels, establishment of interendothelial junctions and formation of a lumen.¹ Angiopoietin 1 (ang-1) stabilizes neovessels by binding to the Tie-2 receptor, thereby affecting junctional molecules¹⁶ and facilitating communication between EC and mural cells.¹⁷ However, an excess of Ang-1 makes vessels too tight and inhibits sprouting. Ang-2 may exert opposing effects (Figure 2): in the absence of VEGF, Ang-2 acts as an antagonist of Ang-1, destabilizes vessels and causes EC death, leading to vessel regression, but it facilitates sprouting in the presence of VEGF.¹⁷

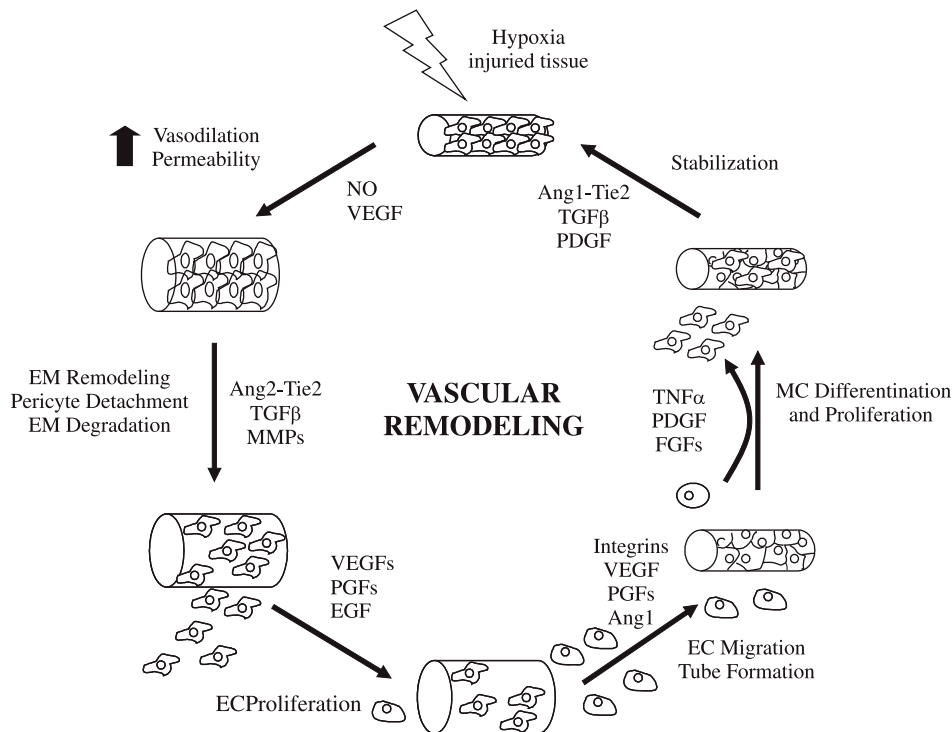


Figure 1. Mechanisms of angiogenesis.

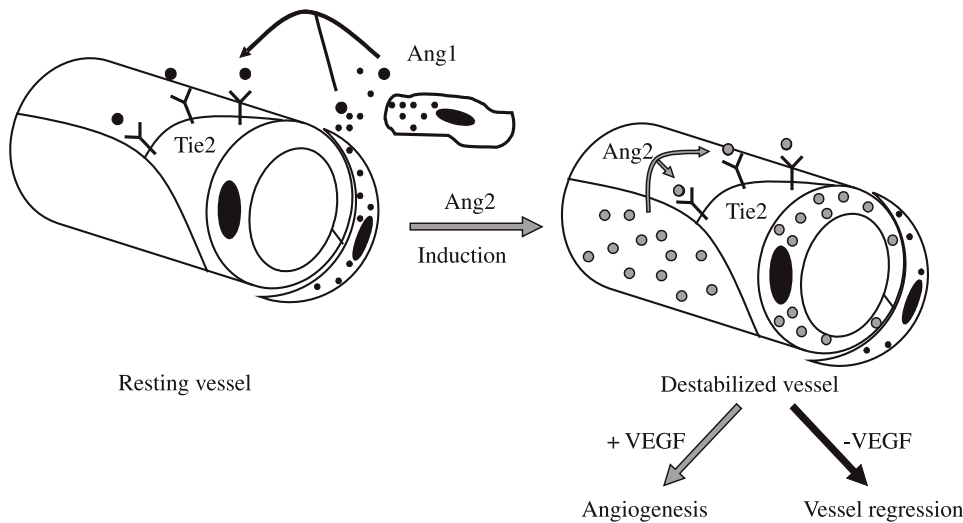


Figure 2. The angiopoietin-Tie2 system in vascular homeostasis and remodeling.

Most molecular mechanisms of angiogenesis are common to the liver and other organs. Physiological hepatic angiogenesis occurs during liver regeneration, leading to the formation of new functional sinusoids, whereas pathological angiogenesis occurs in fibrosis and is characterized by the appearance of capillarized vascular structures. Most chronic liver diseases are characterized by fibrosis and inflammation. During the fibrogenic process, an excessive amount of ECM is synthesized and accumulated. Fibrotic tissue offers resistance to blood flow and to the delivery of oxygen, thus becoming hypoxic. Stimulaton of hypoxia-inducible factors leads to an angiogenic switch, to the upregulation of proangiogenic factors, and to the formation of neovessels.²

Angiogenesis in chronic viral hepatitis

Angiogenesis occurs in the liver of patients infected with hepatitis B or C virus.^{1,2} The pathophysiological significance of chronic viral hepatitis-associated angiogenesis is presently unclear; it has been proposed to exert a beneficial role by contributing to tissue repair and regeneration after liver damage.² It has also been suggested to represent a risk factor for progression to hepatocellular carcinoma in patients with chronic hepatitis C.⁴

The molecular mechanisms involved in chronic viral hepatitis-associated angiogenesis have not been fully identified. Local production of NO as a result of the overexpression of inducible NO synthase in the livers of patients infected with hepatitis C or B virus may participate in the angiogenic response by inducing vasodilatation.^{18,19} VEGF and HGF, whose expression is increased during chronic viral hepatitis,²⁰⁻²² may contribute to enhancing vascular permeability, as suggested by the effect described for these two growth factors. VEGF has been reported to induce NO-mediated vasodilation,¹¹ most probably contributing to the progression of neoangiogenesis.² HGF effects include a decrease and redistribution of

VE-cadherin (which participates in intercellular contacts), and others linking molecules between VE-cadherin and the actin cytoskeleton. Additionally, viral proteins may also play a role in inducing a disruption of interendothelial junctions²³ through mechanisms involving Src kinases, molecules required for vascular permeability during angiogenesis.²⁴ In this way, Sanz-Cameno et al described for the first time the ability of hepatitis B virus (HVB), through HBx protein, to promote Ang-2 expression in liver tissue of chronically infected patients,²⁵ and Lara-Pezzi et al found that matrix metalloproteinase 2 is also up-regulated by HBx protein.²⁶ Binding between EC and ECM may be altered in the livers of chronic viral hepatitis patients.²⁷ Integrin $\alpha v \beta 3$, expressed by activated EC, shows increased tissular expression in chronic hepatitis C (CHC).²² Interestingly, when EC are induced to migrate by HGF, integrin $\alpha v \beta 3$ accumulates at the leading edge,²² which represents the anchoring zone required for pulling the cellular body²⁸ (Table I).

Based on this knowledge, Xalcedo et al²⁹ designed a study in order to test the hypothesis that the evaluation of angiogenesis markers might provide additional, valuable information on the evolution of liver lesion in CHC after antiviral therapy. They included 36 naïve HCV-infected patients and 15 healthy individuals. HCV-infected patients presented a «proangiogenic» profile of angiogenesis soluble markers (VEGF and Ang-2), different from that in healthy controls, while sTie-2 serum levels were similar in both groups. Their results showed a significant correlation between the grade of inflammation in biopsies and serum levels of VEGF, which suggest that inflammation and angiogenesis processes are strongly associated in those patients.

Interestingly, treatment of CHC patients with pegylated interferon plus ribavirin significantly reduced VEGF and Ang-2 levels with respect to pretreatment values. Mean sTie-2 levels were significantly increased after therapy. Taken together, these data indicate that anti-

Table I. Angiogenesis soluble factors with potential to be used as biomarkers.

Factor	Receptor/s	Inhibitors	Effects	Process involved
VEGF family members	Flt-1 Flk-1/KDR	sFlt-1	Increase vascular permeability by loosening intercellular contacts Regulate neovessel lumen formation Induce leucocyte adhesion	Sprouting/budding, vessel growth 3-D organization
Ang-1	Tie-2	sTie-2	Stabilizes intercellular contacts Inhibit permeability	Stabilization of nascent vessels
Ang-2	Tie-2	sTie-2	In the presence of VEGF, it increases capillary diameter and remodels basal lamina In the absence of VEGF, it destabilizes vessels and causes EC death	Vessel regression
aFGF, bFGF, TGF- β , HGF	FGF-R1, FGF-R2 EGF-R, c-Met	Angiostatin endostatin	Induce EC proliferation	Vessel growth
PDGF-BB	PDGF-R β		Recruits pericytes	Stabilization of nascent vessels
Plasminogen activators, MMPs		TIMPs PAI-1	Remodel matrix, release and activate growth factors	ECM remodelling and EC migration
TGF- α	Endoglin		Stimulate extracellular matrix production	Vessel growth

ral combination therapy determines a shift toward an «antiangiogenic» profile of serum markers in CHC patients. The therapy-induced decrease in VEGF levels was significantly correlated with that in ALT and alkaline phosphatase serum levels in responder patients, but not in nonresponders. A similar correlation was found between the decreases in the serum levels of Ang-2 and ALT. These significant relationships suggest some degree of association between angiogenesis and the extent of liver damage (*Figure 1*).

Their novel findings have important implications.²⁹ Firstly, the demonstration of a close relationship between variations in serum levels of angiogenesis markers and response to therapy in CHC patients conceptually supports the clinical role of angiogenesis in chronic inflammatory liver disease. Secondly, the determination of VEGF, Ang-2, and sTie-2 in parallel to the viral load and fibrosis markers may provide complementary information to assess response to treatment and disease progression, which could be helpful to define the optimal follow-up therapeutic strategy for each patient. Further prospective studies with larger cohorts of patients are required to investigate whether these markers may have prognostic value. Additionally, based on these findings, it may be worth investigating in detail the antiangiogenic effects of pegylated interferons and, hypothetically, to consider whether antiangiogenic drugs targeting VEGF or Ang-2 could be of additional value for CHC patients with advanced liver disease and those presently considered as «difficult to treat».

Angiogenesis in autoimmune liver disease

Controversy exists regarding intrahepatic vasculature in autoimmune liver disease. Some authors have reported a tendency to vasopenia and decreased peribiliary capillary plexus in the livers of primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and

autoimmune hepatitis (AIH) patients.^{30,31} This has been attributed to destruction of vascular structures by autoimmune mechanisms, similar to the process undergone by bile ducts in PBC and PSC. However, evidence of angiogenesis has been observed in PBC and AIH. Angiogenesis may occur at a later stage, in response to the hypoxia caused by depletion of existing vessels, although the precise kinetics of the process have not been investigated.

PBC is a chronic inflammatory liver disease of multifactorial etiopathogenesis, characterized by the presence of an intrahepatic mononuclear cell infiltrate, as well as circulating autoantibodies.^{32,33} It has been suggested that immunological mechanisms involving T-lymphocyte-mediated lysis are important in the characteristic bile duct and hepatocellular damage occurring in PBC.³⁴⁻³⁶ Medina et al³⁷ evaluated the phenotype of cellular infiltrates, and the expression of lymphocyte activation, antigen recognition and cell-adhesion molecules in the livers of PBC patients; and they investigated and characterized at the molecular level the pattern of reactivity of proangiogenic factors involved in the formation of neovessels in PBC liver samples. The occurrence of angiogenesis in the livers of PBC patients was a novel finding of this study. CD31 and VE-cadherin positive EC assemble to form new vascular structures, mainly in portal and periportal areas, in association with inflammatory infiltrates and fibrosis. They observed an enhanced expression of VEGF, Ang-1, Ang-2, their receptor Tie-2 and endoglin, which suggests their involvement in EC proliferation and nascent vessels stabilization.

AIH is a chronic progressive liver disease characterized by serological changes (hypergammaglobulinemia, autoantibodies) and interface hepatitis on histological analysis.¹ Tubular structures reflecting formation of new vasculature are observed in inflamed portal tracts of AIH patients.³⁸ As in other inflammatory liver diseases, an up-regulation of inducible NO synthase leading to an en-

hanced production of NO occurs in AIH, and this may participate in angiogenic processes.³⁹ However, information about the mechanisms of angiogenesis in AIH is still very limited.

Angiogenesis in hepatocellular carcinoma

Zhang et al⁴⁰ evaluated the expression of angiogenic factors in hepatocellular carcinoma (HCC) compared with the nontumour liver tissue. They found a high expression of Ang-2 and low expression Ang-1 in HCC samples in comparison with nontumorous tissue, indicating that they play a key role in the carcinogenesis and progression of HCC via angiogenesis. Pappeti and Herman described that tumorous angiogenesis is very different from the physiological process.⁴¹ During this process, vascular quiescence and stabilization are mediated by Ang-1, Ang-2 and Tie-2 system. Therefore, the pathologic state of imbalanced Ang-2/Ang-1 ratio in the presence of VEGF plays a critical role in the transformation of non-cancerous liver to liver cancer by initiating early neovascularization. Vajkoczy et al⁴² reported that tumors in their very early stage are initiated by host vessels via VEGF, VEGF receptor-2 and Ang-2.

In conclusion, expression of Ang-2 against Ang-1 through the Tie2 receptor in the presence of VEGF plays a critical role in initiating early neovascularization and induces transformation of noncancerous liver to HCC.

Conclusions

In recent years, it has become increasingly evident that pathological neoangiogenesis processes occur in chronic inflammatory and fibrotic liver diseases (and probably will be discovered in other nontumoral hepatic alterations). The challenge for the upcoming years is the characterization of the molecular basis and pathways of angiogenic disorders in an integrated manner. The angiogenesis in chronic inflammatory liver diseases seems to have a prognostic value in the evaluation of disease progression. A better understanding of the process may also lead to the design of efficient and safe antiangiogenic therapies using appropriate combinations of inhibitors of angiogenesis. Antiangiogenic therapy is an effective novel treatment for HCC, which is of great clinical significance.⁴³

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