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Review

Pathological Angiogenesis: The New Culprit behind Chronic Liver Disease

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Special Issue: Exploring Novel Treatment Options for Liver Fibrosis: Can Anti-Angiogenics Succeed ?

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Abstract

Angiogenesis is the formation of new blood vessels from the existing ones. It is a complex and highly regulated process which plays a role in a wide variety of physiological and pathological processes. Angiogenesis is essential for a variety of functions in chronic liver disease, including the development and establishment of liver inflammation and fibrosis, the formation of portosystemic collaterals, increase in the splanchnic blood flow, and portal hypertension. Angiogenesis involves a sequence of well-coordinated events which are mediated by a number of strictly regulated interactions between the pro-angiogenic factors and their corresponding receptors expressed on the surface of various vascular (e.g., endothelial cells and pericytes) and stromal components constituting the extracellular matrix. The present review provides an overview of the contribution of angiogenesis to the progression of chronic liver disease, and discusses the functional roles of the key growth factors and cytokines that are known to promote angiogenesis in liver disease, including the vascular endothelial growth factor, placental growth factor, platelet-derived endothelial cell growth factor, and the angiopoietin system. Inhibiting the activity of the aforementioned

factors may serve as a promising therapeutic approach for the treatment of liver disease as these factors may serve as prognostic markers and targets for therapy. The insights into the molecular mechanisms underlying the regulation of the expression of these factors are being generated at an exponential pace, offering novel therapeutic opportunities. One of these insights was the discovery of a novel post-transcriptional mechanism mediated by cytoplasmic polyadenylation element-binding (CPEB) proteins, which regulate the expression of pathological, but not the physiological, vascular endothelial growth factor (VEGF). Essentially, the RNA-binding CPEB proteins also regulate the function of vascular stem/progenitor cells as well as vasculogenesis during the chronic liver disease. The contribution and therapeutic potential of the endogenous inhibitors of angiogenesis have also been discussed in the present review. Finally, promising anti-angiogenic therapeutic strategies applicable in chronic liver disease and liver cancer, including combination therapies, have been stated**.**

Keywords
Angiogenesis; chronic liver disease; vascular endothelial growth factor

1. Introduction

Chronic liver disease is one of the biggest threats to public health, as it affects more than 500 million people throughout the world [1-3]. Every year, more than 1.2 million people lose their lives due to liver cirrhosis [3]. Disturbingly, the incidence of liver disease has been rising dramatically because of increase in the ageing population and the global epidemic of obesity [3-6], both of which are major risk factors and adverse prognostic factors for the chronic liver disease, causing an increase in the mortality rate associated with the disease [7-9]. It is of great concern that 80%–95% of the obese people have non-alcoholic fatty liver disease (NAFLD), which is a major precursor of liver failure, and therefore, a global health challenge [10, 11]. In addition, cirrhosis may eventually progress to hepatocellular carcinoma, which is one of the most prevalent and lethal cancers worldwide and the second most common cause for cancer-related deaths (>600,000 people die of liver cancer each year) [12]. Currently, the only curative treatment option available for advanced chronic liver disease is liver transplantation, which, however, has limitations such as high treatment costs and the scarcity of organ donors. Therefore, there is an urgent requirement of novel strategies that would reduce the burden of liver disease worldwide. In order to achieve that, it is essential to decipher the mechanisms underlying the pathophysiology of chronic liver disease and its progression to liver cancer, most of which unknown to date.

2. Pathological Angiogenesis in Chronic Liver Disease

Angiogenesis is the process of new microvessel formation via sprouting or splitting from pre-existing vessels. After exposure to proangiogenic signals like vascular endothelial growth factor (VEGF), endothelial cells activate, migrate and form new vessels [13-15]. These vessels are then covered by pericytes, attracted by endothelial cell-derived platelet-derived growth factor (PDGF),

providing stabilization and maturation to the neovessels (Figure 1) [16]. Angiogenesis is a pathological hallmark of liver cirrhosis that is closely linked to liver fibrogenesis and inflammation and contributes to increase blood flow in splanchnic organs, to the formation of portosystemic collateral vessels and portal hypertension, playing an important role on disease progression and aggravation (Figure 2) [17-20]. Accordingly, therapeutic strategies aimed at inhibiting angiogenesis could be promising for chronic liver disease [17, 19].

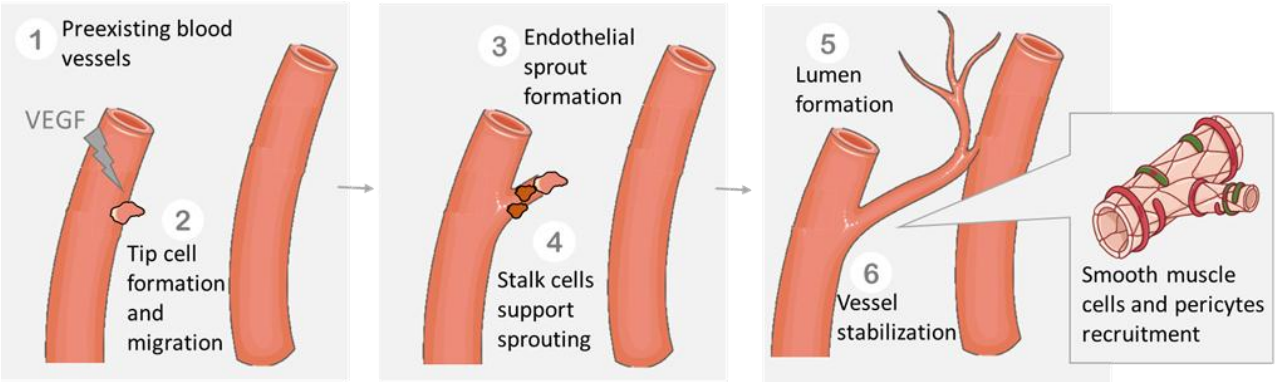

Figure 1 Angiogenesis. Schematic diagram of the angiogenic process. The proangiogenic VEGF is required for the switch from endothelial cell to tip cell (light pink) and the subsequent migration of these cells to form endothelial sprouts. Then, stalk cells (brown) allow the sprouting maintenance followed by lumen formation. Finally, vessel stabilization requires the contribution of smooth muscle cells (red) and pericytes (green). VEGF: Vascular Endothelial Growth Factor.

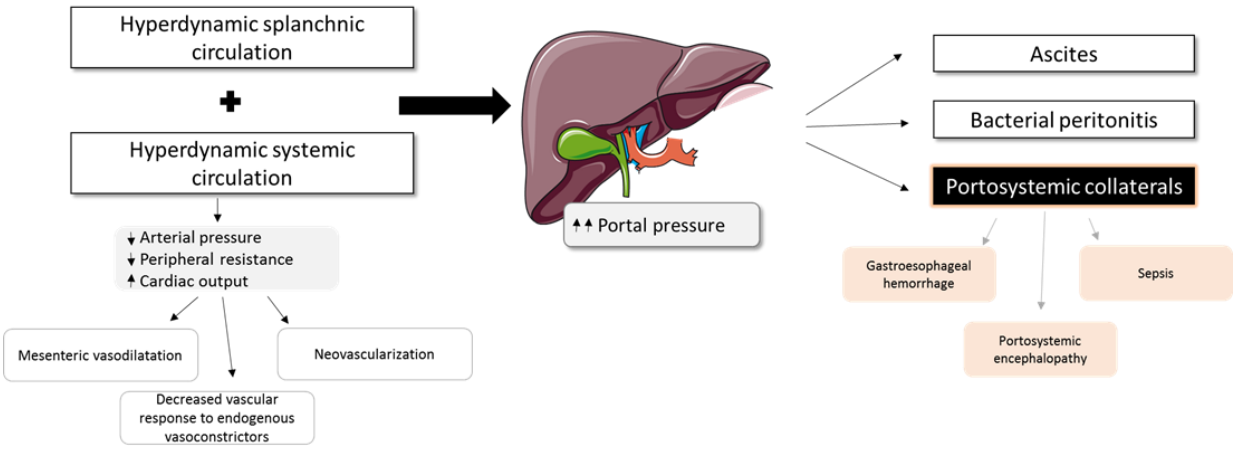

Figure 2 Pathophysiology of chronic liver disease**.** The hyperdynamic systemic circulation reduces arterial pressure and peripheral resistance but increases cardiac output triggering mesenteric arterial vasodilatation, the decrease in vascular response to endogenous vasoconstrictors and neovascularization. General hyperdynamic circulation increases portal pressure and induces the development of ascites, bacterial peritonitis and portosystemic collaterals, which promote gastroesophageal hemorrhage, portosystemic encephalopathy, and sepsis.

2.1 Angiogenesis-Mediated Increase in Splanchnic Blood Flow

Chronic liver disease is characterized by the presence of increased blood flow in splanchnic organs. Since these organs drain into the portal vein, a consequence of enhanced splanchnic blood flow is that portal venous inflow also increases. That in turn perpetuates and exacerbates portal hypertension, promotes ascites and spontaneous bacterial peritonitis, and is associated with formation of portosystemic collateral vessels. These extrahepatic abnormalities are indeed of major importance for chronic liver disease progression and aggravation.

The increase in the splanchnic blood flow in chronic liver disease occurs mainly due to arteriolar vasodilation in the splanchnic organs, which is associated with hyporesponsiveness to endogenous vasoconstrictors, as well as to neovascularization, including angiogenesis and vasculogenesis [17, 19]. All these mechanisms may be driven by similar environmental variables and may lead to splanchnic and systemic hyperdynamic circulations, along with portal hypertension, reduced arterial pressure, peripheral resistance, and increased cardiac output. Therefore, combination therapies acting for the prevention/regression of the newly formed splanchnic vessels by the anti-angiogenic agents as well as for the modulation of vasomotor dynamics by the action of vasoactive substances may have potential clinical relevance in regard to the treatment of chronic liver disease.

2.2. Angiogenesis-Mediated Formation of Portosystemic Collaterals

In chronic liver disease, portosystemic collateral vessels are responsible for life-threatening consequences, including gastro-esophageal hemorrhage, portosystemic encephalopathy, and sepsis. The portosystemic collaterals are formed through the opening and remodeling of pre-existing collaterals, and also through *de novo* formation and maturation of new collateral vessels mediated by active neoangiogenesis [17, 19]. Therefore, targeting angiogenesis might be able to attenuate the development of the collaterals, thereby preventing the formation of large gastro-esophageal varices from small varices.

3. Angiogenic Factors in Chronic Liver Disease

The possible causes of occurrence of angiogenesis in the chronic liver disease include increased shear stress, oxidative stress, hypoxia, inflammation, and endothelial dysfunction, which may, in turn, induce the expression of pro-angiogenic factors which have been described ahead in the report [21-25].

3.1 Vascular Endothelial Growth Factor (VEGF)

VEGF is one of the major pro-angiogenic growth factors driving angiogenesis during cirrhosis (Figure 3). VEGF signaling pathway promotes extensive neovascularization in the liver and mesenteric vascular bed during cirrhosis, increasing the splanchnic blood flow and contributing to liver fibrosis, inflammation, and the development of portosystemic collateral [17, 19]. VEGF and its signaling pathway are switched on at an early stage during the natural history of the chronic liver disease, preceding the increased splanchnic blood flow, and therefore, occurring prior to the enhancement of shear stress [21, 26-28]. This early-stage overexpression of VEGF in the chronic liver disease is post-transcriptionally regulated by the cytoplasmic polyadenylation element-

binding (CPEB) proteins, as described in the later sections of the present report [28]. Blockage of VEGF or its receptor (VEGFR2), using various angiogenesis inhibitors having different modes of action and different prophylactic and therapeutic strategies, effectively decreases the portal pressure as well as mesenteric neoangiogenesis, causes a reduction in the splanchnic blood flow and portosystemic collateralization, and attenuates liver fibrosis [21, 26-29]. In addition, gene therapy utilizing cell-targeted and molecule-targeted liposomal siRNAs directed against endothelial VEGFR2 were reported to markedly ameliorate the development of portosystemic collateral vessels and impair the pathological angiogenic potential of the endothelial cells in a murine model of portal hypertension [30].

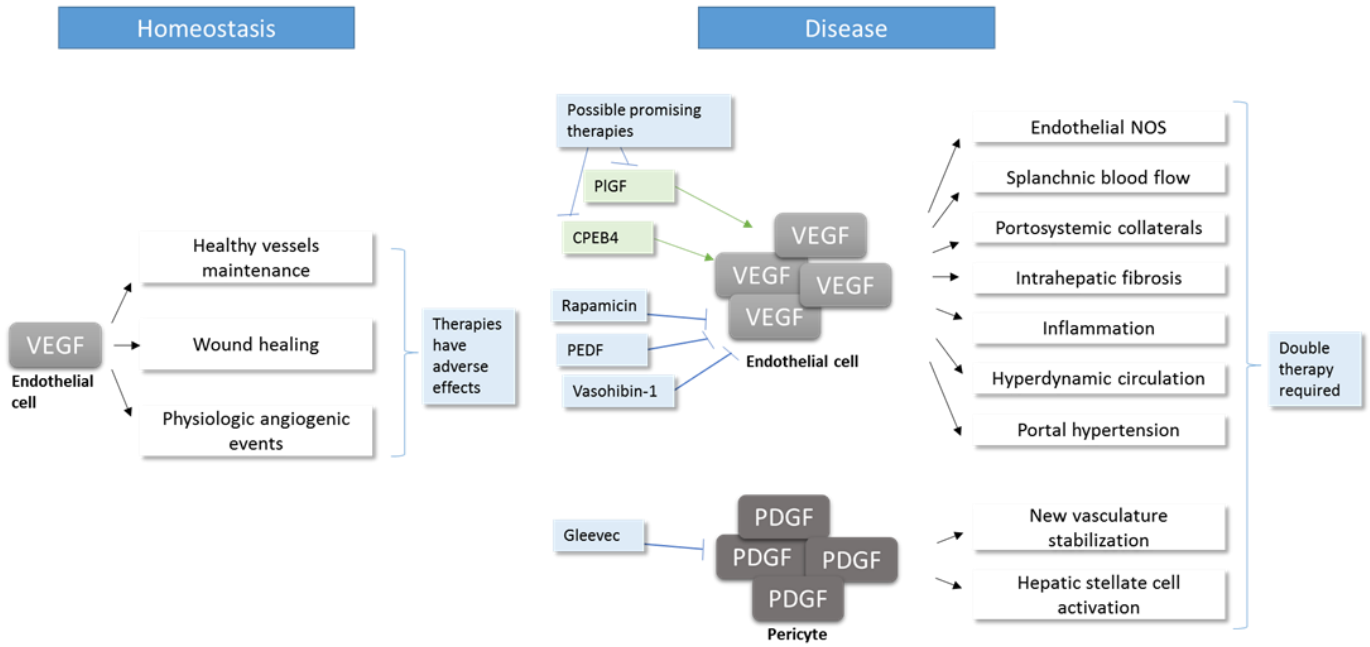

Figure 3 Antiangiogenic therapies. In normal conditions, VEGF is expressed by endothelial cells to maintain vascular homeostasis. Anti-angiogenic therapies have usually adverse effects because they decrease physiologic levels of VEGF. In the disease, endothelial cells secrete excessive VEGF inducing a great variety of events related to tissue damage, inflammation, and liver disease. Pericytes secrete excessive PDGF, which also aggravates the liver disease and new vasculature formation. Therapies inhibiting VEGF and PDGF have shown some positive results but the most promising outcome is derived from combined therapy. VEFG: Vascular Endothelial Factor; PlGF: Placental Growth Factor; NOS: nitric oxide synthase; PEDF: Pigment Epithelium-derived Factor; CPEB4; Cytoplasmic Polyadenylation Element Binding Protein 4; PDGF: Platelet-Derived Growth Factor.

3.2 Placental Growth Factor

Placental growth factor (PlGF) is a pleiotropic cytokine belonging to the VEGF family, which stimulates angiogenesis by binding selectively to a membrane-bound receptor VEGFR1 [31, 32]. PlGF also enhances the activity of VEGF through competitive binding with VEGFR1, displacing VEGF from VEGFR1, thereby allowing the binding of VEGF to VEGFR2, resulting in a complex that has a

stronger tyrosine kinase activity. PlGF also exerts its effects through other mechanisms, such as the intermolecular transphosphorylation of VEGFR-2 following the activation of VEGFR1, which amplifies the VEGFR2 response to VEGF binding. PlGF has attracted great interest as a possible anti-angiogenic therapeutic target, because, unlike VEGF, its activity appears to be dispensable for the physiological angiogenesis and vascular maintenance in healthy adults, while being specifically confined to pathological conditions [33, 34]. The majority of normal mature tissues have been consistently reported to exhibit minimal endogenous PlGF expression, while PlGF is readily upregulated in various diseases, including tumor growth, ischemia, and chronic inflammation, in which PlGF contributes to the functioning of the angiogenic switch that supports pathological vascularization [35, 36]. PlGF might also be playing an important role in liver inflammation, angiogenesis, and fibrosis by promoting the recruitment and activation of hepatic macrophages [37]. In addition, recent studies have demonstrated that PlGF contributes to pathological angiogenesis in the lung during hepatopulmonary syndrome [38], which is an important and a relatively common clinical complication of cirrhosis [39, 40].

3.3 Platelet-Derived Growth Factor

The expression of platelet-derived growth factor (PDGF) and its PDGFRβ receptor is upregulated during chronic liver disease in mesentery and cirrhotic liver and plays a major role in the excessive neovascularization of these vascular beds. During angiogenesis, endothelial cells secrete PDGF and, thereby, paracrinically stimulate the recruitment of PDGFRβ-positive pericytes, which, in turn, act as supportive vascular smooth muscle cells, thereby stabilizing the vascular architecture of the nascent vessel. Interestingly, a combined antiangiogenic treatment directed against endothelial cells and pericytes, using VEGF and PDGF inhibitors simultaneously, reduces the hemodynamic abnormalities of chronic liver disease [26]. This is due to the fact that removal of pericyte coverage by anti-PDGF molecules leads to exposed endothelial tubes, making endothelial cells more susceptible and vulnerable to anti-VEGF treatment. In this regard, targeting VEGFR-2 and PDGFR-β signalling pathways with sorafenib [41] significantly reduces portosystemic collateralization, hyperdynamic splanchnic circulation, intrahepatic fibrosis and portal pressure in experimental cirrhosis [18, 20, 42], with potential beneficial effects also in humans [43].

3.4 Angiopoietin/Tie System

Angiopoietins and their corresponding tyrosine kinase receptors have been recognized as crucial cytokines that govern angiogenic growth and remodeling. In addition to their established functions in developmental and pathological angiogenesis, angiopoietins serve as a link between angiogenesis and inflammation. The levels of angiopoietin-2 have been reported to be increased in patients with cirrhosis, with the increased levels being correlated with disease prognosis [44, 45]. The therapeutic inhibition of angiopoietin-2 has been reported to reduce inflammation and fibrosis in the experimental models of liver fibrosis induced by carbon tetrachloride [46] or those of non-alcoholic steatohepatitis (NASH) [47]. In the latter study, the progression to hepatocellular carcinoma was observed to decrease as well.

3.5 Endogenous Inhibitors of Angiogenesis

Pigment epithelium-derived factor (PEDF) is one of the most powerful endogenous inhibitors of angiogenesis. The expression of PEDF is unidirectionally increased along with VEGF in mesentery and liver in the experimental models of cirrhosis [48, 49]. This upregulation of PEDF occurs as an attempt to counteract the pro-angiogenic activity of VEGF. In this context, overexpression of PEDF through adenovirus-mediated gene transfer was reported to create an imbalance between VEGF and PEDF in favor of inhibition of angiogenesis, inducing portal pressure and causing a partial correction of excessive angiogenesis, in experimental cirrhosis [48, 49]. Exogenous PEDF supplementation during the early phase of cirrhosis could, therefore, serve as a promising therapeutic approach to prevent disease progression. Another important endogenous inhibitor of angiogenesis is vasohibin-1, which is selectively induced by VEGF through a specific mechanism involving a negative-feedback loop. The overexpression of vasohibin-1 through an adenovirus-mediated gene transfer in cirrhotic rats was reported to disrupt the VEGF-vasohibin-1 negative-feedback loop, bringing the pathologically overexpressed VEGF to normal levels, although not allowing the levels to reach below normal and maintaining a baseline amount of VEGF required for the homeostasis of healthy vessels. As a consequence, liver fibrosis, portosystemic collateral vessel formation, and portal pressure were reported to be significantly attenuated in the treated cirrhotic rats [50, 51].

3.6 Cytoplasmic Polyadenylation Element-Binding (CPEB) Proteins: Critical for Pathological Angiogenesis and Vasculogenesis

CPEB proteins are the RNA-binding proteins that regulate the translation of a specific group of mRNAs characterized by the presence of certain specific sequences, known as cytoplasmic polyadenylation elements (CPEs), in their non-coding 3'-untranslated regions (3'UTR) [52-54]. One of these CPEB-regulated mRNAs is the VEGF mRNA [28]. Upon the induction of cirrhosis, there is a rapid upregulation and activation of Aurora kinase-A through autophosphorylation in mesentery and liver. The activated Aurora kinase-A, in turn, phosphorylates and activates CPEB1 [55-59], which then promotes alternative nuclear processing in the 3'UTR regions of the VEGF and CPEB4 mRNAs, resulting in the deletion of translation repressor elements. Therefore, CPEB1 "takes off the brakes" for CPEB4 mRNA and the resulting CPEB4 overexpression promotes cytoplasmic polyadenylation of VEGF mRNA, increasing its translation and generating high levels of VEGF [28]. Essentially, this CPEB-mediated regulatory mechanism is crucial for pathological angiogenesis and dispensable for the physiological neovascularization. Additionally, CPEB4 is required for the proliferation of progenitor stem cells present in the vascular walls of blood vessels; these cells are able to differentiate and mediate the abnormal growth of new vessels during liver disease [60]. In both cases (angiogenesis and vasculogenesis), interference with the CPEB4 protein disrupts only the formation of pathological vessels, while positive vascularization remains intact. When considered together, these findings reinforce the potential of CPEB4 as a therapeutic target for the treatment of liver cirrhosis, as well as potentially for the other angiogenesis-dependent diseases, such as inflammatory disorders and cancer.

4. Anti-Angiogenic Therapeutic Strategies and Targets

Angiogenesis and angiogenic growth factors may serve as promising therapeutic targets and potential diagnostic markers for chronic liver disease, as described in the earlier sections. These have been already used in clinical settings for the treatment of hepatocellular carcinoma. Since there are different steps involved in the process of angiogenesis and these steps are dependent on various molecules, the most suitable approach to target angiogenesis in most of the patients is the simultaneous administration of a combination of different inhibitors.

4.1 Tyrosine Kinase Inhibitors

The antiangiogenic drug that has been used the most is sorafenib, which inhibits receptor and non-receptor tyrosine kinases (TK). Sorafenib achieves its anti-angiogenic and anti-tumor response through the suppression of Raf/MEK/ERK signaling pathway and the blocking of VEGF and PDGF among other molecules [61]. Several studies have confirmed the effectiveness of sorafenib in inflammation, fibrosis, and angiogenesis, in the animal models of cirrhosis [18, 62]. It is noteworthy that it is possible to activate most of the hepatic cells orchestrating the pathophysiology of liver injury, such as the hepatic stellate cells and Kupffer cells, through the binding of pro-angiogenic factors to their corresponding receptors present on the surface of these cells. The treatment with the inhibitors of angiogenesis, such as sorafenib, has been consistently reported to exhibit multiple beneficial effects, decreasing only the pathological neovascularization as well as attenuating liver inflammation and fibrosis [18]. Sorafenib in combination with propranolol has been demonstrated to reduce portal pressure in cirrhotic rats and to improve intrahepatic and extrahepatic complications in liver disease [63]. Branivib is another multi-target tyrosine kinase inhibitor reported in the literature for the treatment of hepatocellular carcinoma. Branivib is specific for VEGFR and the fibroblast growth factor receptor (FGFR) and is commonly used for its anti-tumor effect in the patients exhibiting resistance to sorafenib [64]. Cabozantinib has also been reported to achieve good results in the patients exhibiting resistance to sorafenib, through the blocking of both MET and VEGFR2 [65]. Another usual agent is Imatinib (Gleevec), which is an inhibitor of PDGF that has provided the best results in the treatment of advanced hepatocellular carcinoma when used in combination with somatostatin [66]. Imatinib in combination with VEGF inhibitors has also been demonstrated to reverse portal hypertension and hyperdynamic splanchnic circulation in rats [26]. Other TK inhibitors have also been reported for the treatment of liver cancer, such as sunitinib, lenvatinib, linifanib, and nintedanib [67]. The beneficial effects of sunitinib have also been demonstrated in cirrhotic rats [20].

4.2 Statins

Statins specifically target the liver sinusoidal endothelial cells and the hepatic stellate cells via Krüppel-like factor 2 (KLF2), which exhibits increased levels in both the types of cells and regulates the cell and tissue growth at the same time, reducing pro-angiogenic signaling pathways through HIF1α inhibition [68]. It is required for physiological vasculature development and for impeding the malformation of vessels, thereby ameliorating fibrosis, and as a consequence, portal hypertension.

4.3 Other Anti-Angiogenic Therapeutic Strategies

Other angiogenesis modulators that have demonstrated promising results in the context of liver disease are as follows: somatostatin analogues [66, 69], anti-oxidant drugs [24], inhibitors of heme oxygenase [23], mTOR inhibitors [26], 2'-hydroxyflavanone [70], thalidomide [71], N-acetylcysteine [72], polyphenols [73], pioglitazone [74], and the histone deacetylase inhibitor largazole [75].

5. Conclusion

Angiogenesis is crucial for the major processes associated with chronic liver disease, including fibrogenesis, inflammation, portosystemic collateralization, and portal hypertension. Improved understanding of the molecular and cellular mechanisms underlying the pathological angiogenesis is essential for advancements in the treatment strategies for the associated diseases. This is of particular significance as there are limited treatment options available for the patients suffering from chronic liver disease.

Table 1 Anti-angiogenic therapeutic strategies used in chronic liver disease and liver cancer.

Drug	Effect	Reference
Sorafenib	Tyrosine kinase inhibitor that suppresses Raf/MEK/ERK signaling pathway and blocks VEGF and PDGF	[18, 61, 62]
Branivib	Multi-target tyrosine kinase inhibitor targets VEGFR and FGFR	[64]
Cabozantinib	Blocks MET and VEGFR2	[65]
Imatinib	Inhibits PDGF in HSC	[66]
Sunitinib	Tyrosine kinase inhibitor	[67]
Lenvatinib	Tyrosine kinase inhibitor	[67]
Linifanib	Tyrosine kinase inhibitor	[67]
Nintedanib	Tyrosine kinase inhibitor	[67]
Bevacizumab	Targets VEGF	[67]
Erlotinib	Tyrosine kinase inhibitor	[67]
Simvastatin	Downregulates proangiogenic signals by KLF2 increase in LSEC and HSC	[68]
Somatostatin Octreotide	Angiogenesis modulator	[66, 69]
Rapamycin	mTOR inhibitor	[26]
2'Hydroxyflavanone	Angiogenesis modulator	[70]
Thalidomide	Angiogenesis modulator	[71]
N-acetylcysteine	Angiogenesis modulator	[72]

Author Contributions

The work and the content were proposed by Mercedes Fernandez. Marta Ramirez and Mercedes Fernandez performed the literature review and wrote the manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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