Editorial

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# Endothelial progenitor cells in cirrhosis: The more, the merrier?

Pierre-Emmanuel Rautou*

*Service d'Hépatologie, Hôpital Beaujon, Assistance Publique-Hôpitaux de Paris, Clichy, France; INSERM, U970, Paris Cardiovascular Research Center – PARCC, Paris, France; Université Paris Descartes, Sorbonne Paris Cité, UMR-S970, Paris, France; INSERM, U773, Centre de Recherche Biomédicale Bichat-Beaujon CRB3, Clichy, France; Université Denis Diderot-Paris 7, Paris, France*



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Portal hypertension is a severe and frequent complication of chronic liver diseases. Its consequences represent a leading cause of death or of liver transplantation in patients with cirrhosis. Its pathogenesis mainly originates from an elevation in hepatic vascular resistance to portal blood flow due to structural and dynamic factors. Structural factors include fibrosis, vascular remodeling with capillarization of sinusoids, vascular occlusion, and regenerative nodule formation. The dynamic component is due to increased hepatic vascular tone, related to reduced bioavailability of intrahepatic vasodilators, particularly of nitric oxide, and increased activity of several endogenous vasoconstrictors [1]. Secondarily to the increased hepatic vascular resistance, there is a progressive splanchnic arterial vasodilatation, that increments portal blood flow, and aggravates and perpetuates portal hypertension.

In recent years, angiogenesis, a dynamic process leading to the formation of new vessels from pre-existing blood vessels [2], has been linked to both intrahepatic and extrahepatic components of portal hypertension [2,3]. Within the liver, angiogenesis is postulated to contribute to portal hypertension by promoting fibrogenesis. Indeed, angiogenesis is a typical feature of liver fibrosis. Moreover, pharmacologic interventions that inhibit angiogenesis, especially using receptor tyrosine kinase inhibitors such as sorafenib or sunitinib, or molecules targeting vascular endothelial growth factor (VEGF) receptor type 2 or Tie2, induce a decrease in hepatic fibrosis [2]. Outside of the liver, angiogenesis contributes to the formation of portosystemic collaterals and the increase in splanchnic blood flow [3]. Portal hypertension is characterized by an extensive network of portosystemic collateral vessels, which include the esophageal and gastric varices. Evidence supporting a role for extrahepatic angiogenesis in the pathogenesis of portal hypertension comprises recent investigations demonstrating that VEGF, a potent angiogenic factor, is overexpressed in splanchnic organs from portal hypertensive animals. The expression of VEGF receptor-2 and the endothelial cell marker CD31 is also increased in the splanchnic territory in experimental models of portal hypertension. Importantly, blockade of VEGF-derived signaling markedly decreases splanchnic vascularization and portal venous inflow in rodent models of portal hypertension [1,3].

In this issue of the *Journal of Hepatology*, Kaur and colleagues reported increased levels of circulating endothelial progenitor cells (EPC) in cirrhosis patients and showed that these cells stimulated angiogenesis *in vitro* (Fig. 1) [4]. This study represents an important step forward in the understanding of the pathogenesis of intrahepatic angiogenesis in cirrhosis and suggests a specificity of cirrhotic EPCs.

EPCs are immature precursor cells, smaller than 15 μm that are detectable in blood and bone marrow [5,6]. They are rare since their concentration in blood is around 0.005–0.01% of the white blood cells [5]. *In vitro*, EPCs are characterized by their ability to form adherent colonies that proliferate and differentiate into endothelial lineage. *In vivo*, they contribute to angiogenesis within ischemic sites or to vascular repair after vessel wall injury [6]. Nevertheless, accumulating data demonstrate that these properties identify a heterogeneous pool of cells with regard to their origin, differentiation and functional characteristics, as reviewed elsewhere [5]. In the absence of an unambiguous and consistent definition of EPCs, these cells are typically identified either by surface antigens using flow cytometry, or by *in vitro* colony-forming assays. Using flow cytometry, the minimal antigenic profile should include one or more marker of immaturity (usually CD34 and/or CD133 in humans; CD34, c-kit, or Sca-1 in mice), and one or more marker of endothelial commitment (usually VEGF receptor-2). The alternative approach, i.e., colony formation in culture, measures not only absolute numbers of EPCs, but also their overall proliferative capacity and may be more reflective of EPC capacity for endothelial repair.

Kaur and colleagues used both assays and observed higher circulating EPC levels in chronic liver disease patients without hepatocellular carcinoma (HCC) than in controls. This increase was more pronounced in cirrhosis patients and inversely correlated with prothrombin time. Likewise, another group of

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* Address: Service d'Hépatologie, Hôpital Beaujon, 92 110 Clichy, France. Tel.: +33 1 40 87 55 19; fax: +33 1 40 87 44 26.

*E-mail address:* pierre-emmanuel.rautou@inserm.fr

*Abbreviations:* EPC, endothelial progenitor cell; HCC, hepatocellular carcinoma; VEGF, vascular endothelial growth factor.

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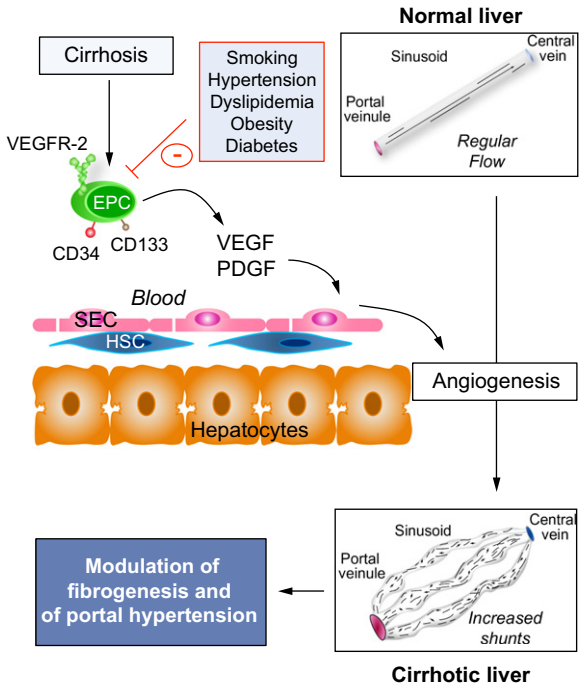


**Fig. 1. Proposed mechanism of endothelial progenitor cells (EPCs) regulation of angiogenesis in cirrhosis**. Cardiovascular risk factors are associated with reduced circulating EPC levels. Conversely, circulating EPC levels are increased in cirrhosis patients. EPCs are likely recruited into the liver were they release the growth factors VEGF and PDGF. This stimulates SEC proliferation and formation of new vessels in the liver. Whether these new vessels eventually increase or reduce liver fibrosis and portal hypertension remains to be determined. (Adapted from Thabut and Shah [2]). EPC, endothelial progenitor cell; HSC, hepatic stellate cell; PDGF, platelet-derived growth factor; SEC, sinusoidal endothelial cells; VEGF, vascular endothelial growth factor.

investigators previously found a borderline significant increase in circulating EPC levels in cirrhosis patients without HCC [7]. Interestingly, EPC levels correlated with hepatic venous pressure gradient [7]. Three other studies reported either no difference or lower circulating EPC levels in cirrhosis patients as compared to controls [8–10]. Several factors can account for this discrepancy. First, controls were not matched for cardio-vascular risk factors in any of the above mentioned studies. It limits the interpretation of the results since absolute number and functional capacity of EPCs are reduced in individuals with diabetes, smoking, arterial hypertension, hypercholesterolemia, obesity or aging [5,6]. Some of these risk factors, such as diabetes and smoking, are frequent in cirrhosis patients and may mask the impact of cirrhosis on EPC levels. For instance, in the study by Chen and colleagues, 52% and 31% of cirrhosis patients were smokers and had diabetes, respectively. This prevalence was not mentioned in controls but we can assume that it was lower [8]. Second, these three studies likely suffer from a limitation in power since they included only 10–16 cirrhosis patients without HCC [8–10]. HCC patients should be analyzed separately as HCC is associated with an increase in circulating EPC levels [7,9,11]. Third, these studies did not mention the severity of cirrhosis in patients without HCC, while circulating EPC levels seem to be increased primarily in advanced cirrhosis patients [8–10]. Altogether, these data suggest that circulating EPC levels are increased in patients with advanced cirrhosis. Their potential interest as biomarkers of fibrosis and/or portal hypertension remains to be assessed in large cohorts of patients with controls matched for cardiovascular risk factors.

The mechanism responsible for this increase in circulating EPCs levels in cirrhosis is unknown. If acute ischemia generally increases circulating EPC levels, reduced levels and/or function are reported in patients with chronic cardiovascular diseases, such as stable coronary artery disease, congestive heart failure, cerebrovascular disease, transplant vasculopathy or stent restenosis [6]. This suggests that chronic liver ischemia potentially associated with cirrhosis may not account for elevated circulating EPC levels in cirrhosis [12]. Mobilization of EPCs depends on the nitric oxide-dependent upregulation of matrix metalloproteinase-9 expression by bone marrow stromal cells [13]. A large body of evidence unmasks extrahepatic overproduction of nitric oxide as one of the hallmarks of cirrhosis [14]. We can speculate that this high nitric oxide production could contribute to EPC mobilization and to elevated EPC levels in cirrhosis.

Kaur and colleagues demonstrated that circulating EPCs from cirrhosis patients stimulated proliferation of sinusoidal endothelial cells and their organization in tubular structures. This effect was dependent upon the release by cirrhotic EPCs of two proangiogenic growth factors: platelet-derived growth factor and VEGF. Given the above mentioned postulated contribution of hepatic angiogenesis to fibrogenesis, the authors suggested that circulating EPCs may contribute to cirrhosis progression and its complications. In contrast, several previous studies demonstrated that injection of healthy rat EPCs into cirrhosis rats decreased liver fibrosis and inflammation, increased hepatocyte proliferation and improved liver function [15–18]. Importantly, EPC injection also induced intrahepatic angiogenesis, decreased portal pressure and increased hepatic blood flow [16]. If these data confirm the proangiogenic effect of EPCs in the cirrhotic liver, they question the view that liver angiogenesis induced by EPCs contributes to fibrosis and portal hypertension. Two hypotheses can reconcile these observations. First, the effect of EPCs on liver fibrosis and portal hypertension may depend upon their source. Kaur and colleagues found a two to threefold higher proangiogenic activity with circulating EPCs from cirrhosis patients than from healthy controls [4]. All studies reporting a favorable effect of EPC transplantation used EPCs derived from the bone marrow of healthy rats. This suggests that the proangiogenic activity of cirrhotic EPCs may be excessive and induce the formation of abnormal vessels, thus worsening liver fibrosis and portal hypertension. Alternatively, liver angiogenesis induced by cirrhotic EPCs may be beneficial and the increase in EPC levels in cirrhosis may be a compensatory mechanism. In support of this statement, EPC are generally implicated in repair to injury response [6]. Moreover, new intrahepatic vessels could theoretically act as portal hypertension decompressing shunts [2]. Finally, beside the studies mentioned above showing that therapeutic approaches targeting aberrant vasculature structure in cirrhosis could have a beneficial effect on portal hypertension, few others did not. For example, in a study performed using two complementary models of cirrhosis, the administration of cilengitide, an inhibitor of the $\alpha v\beta 3$ integrin that plays an important role in liver angiogenesis, promoted hepatic fibrosis and inflammation despite its anti-angiogenic effects [19]. Assessing the effect of the transplantation of cirrhotic EPCs into cirrhosis rats would help determine the correct hypothesis.

In conclusion, this study not only highlights a new mechanism for intrahepatic angiogenesis but also provides new prospects. The proangiogenic effect of EPCs in cirrhosis may not be restricted to liver sinusoidal endothelial cells. EPCs may also contribute to splanchnic angiogenesis, which plays a role in the development and maintenance of splanchnic hyperemia in portal hypertension [1]. Moreover, in different population of subjects, circulating EPC levels correlated with endothelial function [5,20]. Whether EPC could contribute to the arterial vasodilation associated with portal hypertension in cirrhosis patients remains to be determined. Further studies are thus needed to delineate how EPCs may modulate portal hypertension.

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**Conflict of interest**

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