THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Liver sinusoidal endothelial cells in disease - And for therapy?

OPEN ACCESS

Liver sinusoidal endothelial cells in disease – And for therapy?

Benjamin M. Stutchfield, Stuart J. Forbes*

Medical Research Council Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK

COMMENTARY ON:

Role of differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic fibrosis in rats. Xie G, Wang X, Wang L, Wang L, Atkinson RD, Kanel GC, Gaarde WA, Deleve LD. Gastroenterology. April 2012;142(4):918–927.e6. Copyright (2012). Abstract reprinted by permission from the American Gastroenterological Association.

http://www.ncbi.nlm.nih.gov/pubmed/22178212

Abstract: Background & Aims: *Capillarization, characterized by loss of differentiation of liver sinusoidal endothelial cells (LSECs), precedes the onset of hepatic fibrosis. We investigated whether restoration of LSEC differentiation would normalize crosstalk with activated hepatic stellate cells (HSC) and thereby promote quiescence of HSC and regression of fibrosis.*
Methods: *Rat LSECs were cultured with inhibitors and/or agonists and examined by scanning electron microscopy for fenestrae in sieve plates. Cirrhosis was induced in rats using thioacetamide, followed by administration of BAY 60-2770, an activator of soluble guanylate cyclase (sGC). Fibrosis was assessed by Sirius red staining; expression of α-smooth muscle actin was measured by immunoblot analysis.*
Results: *Maintenance of LSEC differentiation requires vascular endothelial growth factor-A stimulation of nitric oxide-dependent signaling (via sGC and cyclic guanosine monophosphate) and nitric oxide-independent signaling. In rats with thioacetamide-induced cirrhosis, BAY 60-2770 accelerated the complete reversal of capillarization (restored differentiation of LSECs) without directly affecting activation of HSCs or fibrosis. Restoration of differentiation to LSECs led to quiescence of HSCs and regression of fibrosis in the absence of further exposure to BAY 60-2770. Activation of sGC with BAY 60-2770 prevented progression of cirrhosis, despite continued administration of thioacetamide.*
Conclusions: *The state of LSEC differentiation plays a pivotal role in HSC activation and the fibrotic process.*

And

Liver sinusoidal endothelial cell progenitor cells promote liver regeneration in rats. Wang L, Wang X, Xie G, Wang L, Hill CK, DeLeve LD. J Clin Invest. April 2 2012;122(4):1567–1573. Copyright (2012). Abstract reprinted by permission from the American Society for Clinical Investigation.

http://www.ncbi.nlm.nih.gov/pubmed/22406533

Abstract: *The ability of the liver to regenerate is crucial to protect liver function after injury and during chronic disease. Increases in hepatocyte growth factor (HGF) in liver sinusoidal endothelial cells (LSECs) are thought to drive liver regeneration. However, in contrast to endothelial progenitor cells, mature LSECs express little HGF. Therefore, we sought to establish in rats whether liver injury causes BM LSEC progenitor cells to engraft in the liver and provide increased levels of HGF and to examine the relative contribution of resident and BM LSEC progenitors. LSEC label-retaining cells and progenitors were identified in liver and LSEC progenitors in BM. BM LSEC progenitors did not contribute to normal LSEC turnover in the liver. However, after partial hepatectomy, BM LSEC progenitor proliferation and mobilization to the circulation doubled. In the liver, one-quarter of the LSECs were BM derived, and BM LSEC progenitors differentiated into fenestrated LSECs. When irradiated rats underwent partial hepatectomy, liver regeneration was compromised, but infusion of LSEC progenitors rescued the defect. Further analysis revealed that BM LSEC progenitors expressed substantially more HGF and were more proliferative than resident LSEC progenitors after partial hepatectomy. Resident LSEC progenitors within their niche may play a smaller role in recovery from partial hepatectomy than BM LSEC progenitors, but, when infused after injury, these progenitors engrafted and expanded markedly over a 2-month period. In conclusion, LSEC progenitor cells are present in liver and BM, and recruitment of BM LSEC progenitors is necessary for normal liver regeneration.*

The non-parenchymal cells of the liver have been receiving greater prominence in the setting of injury and regeneration. Hepatic stellate cells (HSCs) are the archetypal scar forming cells of the liver but are also known for their pro-regenerative capabilities and are able to secrete hepatocyte growth factor (HGF) and promote liver regeneration [1]. Activated HSCs, myofibroblasts can also promote biliary regeneration from hepatic progenitor
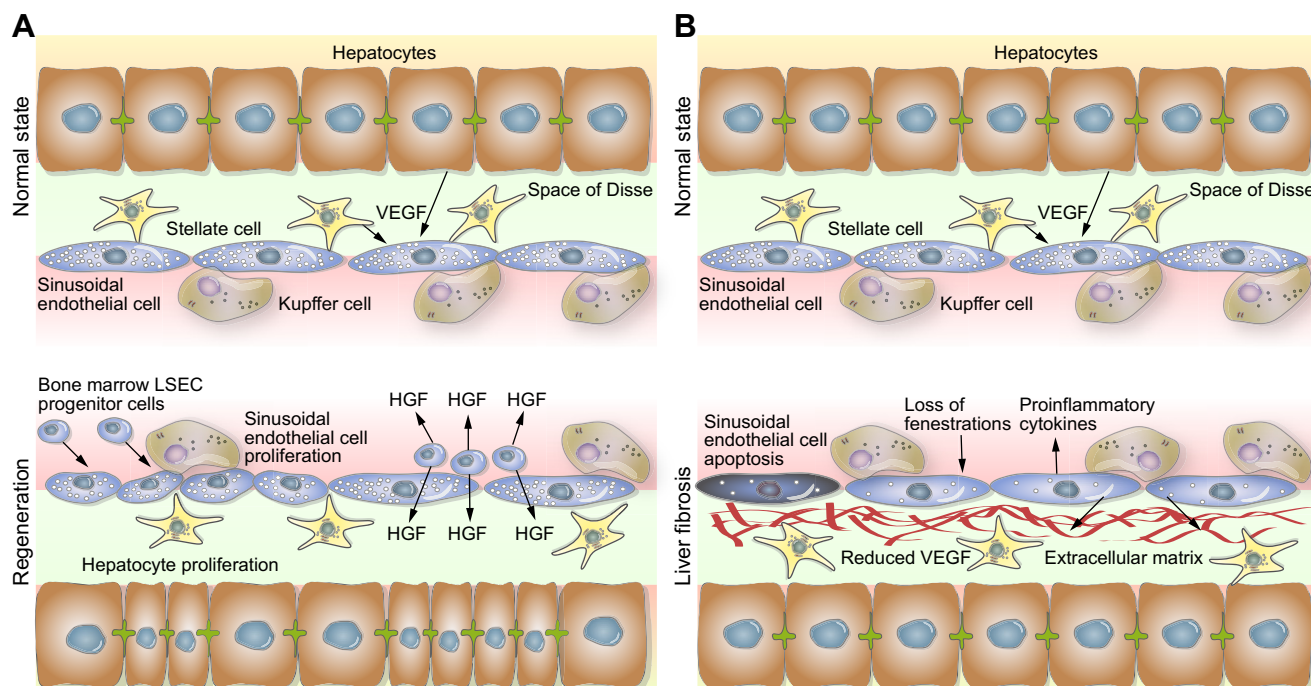
Fig. 1. The endothelial cells of the hepatic sinusoid in health, fibrosis, and regeneration. In the normal liver, LSECs are fenestrated and are flanked by stellate cells and Kupffer cells. In fibrosis, fenestrae are lost and stellate cells are activated. In liver regeneration following hepatectomy, LSEC progenitors from the BM engraft the liver, divide and express HGF strongly, which promotes hepatocyte proliferation. HGF, hepatocyte growth factor.

cells (HPCs) in chronic liver injury through the secretion of Notch ligands [2]. Likewise hepatic macrophages are now recognized to have an important role in both the promotion and regression of liver fibrosis [3]. In liver regeneration following partial hepatectomy, macrophages facilitate regeneration through the secretion of IL-6 [4] and in chronic liver injury promote the formation of hepatocytes from HPCs through Wnt secretion [2]. In development, endothelial cells have a role in organogenesis [5] and so it is not surprising that liver sinusoidal endothelial cells (LSECs) also have a role in liver regeneration. This was confirmed in an elegant paper from the group of Rafii who showed that LSECs are essential for efficient liver regeneration in a manner dependent upon the DNA binding protein inhibitor Id1 in endothelial progenitor cells [6]. Two recent interesting papers from the group of Deleve have examined the role of LSECs and their precursors in liver fibrosis [7] and regeneration [8]. Pathological observations in both humans and rodents have shown so called LSECs capillarization in response to injury. This change occurs prior to the development of fibrosis and is characterized by loss of LSEC fenestrations and the formation of a basement membrane. Xie *et al.* demonstrate that maintenance of LSEC phenotype is dependent upon vascular endothelial growth factor A (VEGF) driving both nitric oxide-dependent signaling (via soluble guanylate cyclase (sGC)) and nitric oxide-independent signaling [7]. The *in vivo* relevance of these findings was shown in the thioacetamide model of liver fibrosis where the capillarization was reversed through the use of a compound which activated sGC. This prevented progression of fibrosis despite on-going injury and accelerated fibrosis regression in recovery. This important study adds to the collection of cells in the liver directly implicated in the formation of liver fibrosis and provides a further cellular target for antifib-

rotic strategies, although, as this is a new area, it may be worth considering whether capillarization has any positive roles. Whilst morphological changes in LSECs are undoubtedly important in the formation of liver fibrosis, there are likely to be a wealth of further signaling mechanisms at play also between LSECs, HSCs, and even macrophages (Fig. 1).

Another feature of LSECs is that they respond to partial hepatectomy by proliferating and secreting the hepatocyte mitogen HGF. However, mature LSECs are typically low in HGF, while high levels of HGF are seen in the more generic endothelial progenitor cell and bone marrow-derived LSEC progenitors [8]. In the second paper, the group use a rat model of partial hepatectomy to demonstrate that LSEC progenitor cells arise from both the liver and bone marrow (BM) to contribute to the regenerative response [8]. These mobilized BM LSEC progenitors engrafted in the liver, proliferated and were the highest secretors of HGF. At a functional level, blocking BM LSEC progenitor mobilization by limb irradiation reduced the rate of liver regeneration. This could be reversed by injecting intravenously BM and liver-derived LSEC progenitor cells, which could engraft the liver and form fenestrated LSECs. It is worth speculating whether this mobilization of LSEC progenitors is related to the increased HGF expression seen in lung, kidney, and spleen, contemporaneous to that in the liver following partial hepatectomy. Whilst the HGF secreting role of LSECs is indeed important, there are again likely to be many other mechanisms to this pro-regenerative function.

These two studies open up a number of interesting, clinically relevant questions, and possibilities. In the clinical setting, can failure of effective liver regeneration result from hepatic LSEC dysfunction or from a failure of mobilization of BM LSECs? Could future liver remnant volume be enhanced in the context of portal

vein embolization or following extended hepatectomy? Chemotherapy can injure LSECs, risking sinusoidal obstructive syndrome and impaired regeneration following resection of hepatic metastases [9]. Could modulation of intrahepatic LSECs promote liver regeneration in this context? Nakamura *et al.* have tested endothelial progenitor cells (EPCs) as a cellular therapy for fibrosis. Injections of syngeneic EPCs in a rat model of liver cirrhosis reduced stellate cell activation, MMPs were increased and fibrosis was reduced, along with a stimulation of hepatocyte proliferation [10]. Whilst promising, the clinical development of such cell therapies may yet be challenging. If an autologous approach were taken, it is not clear if therapeutically relevant numbers of LSEC progenitors could be mobilized or isolated from patients with clinically significant liver dysfunction and indeed whether they would be functionally competent. Whilst logistical hurdles remain, the recent publications certainly suggest an area worthy of further study.

Financial support

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Malik R, Selden C, Hodgson H. The role of non-parenchymal cells in liver growth. Semin Cell Dev Biol 2002;13:425–431.

[2] Boulter L, Govaere O, Bird TG, Radulescu S, Ramachandran P, Pellicoro A, et al. Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. Nat Med 2012;18:572–579.

[3] Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 2005;115:56–65.

[4] Aldeguer X, Debonera F, Shaked A, Krasinkas AM, Gelman AE, Que X, et al. Interleukin-6 from intrahepatic cells of bone marrow origin is required for normal murine liver regeneration. Hepatology 2002;35:40–48.

[5] Matsumoto K, Yoshitomi H, Rossant J, Zaret KS. Liver organogenesis promoted by endothelial cells prior to vascular function. Science 2001;294:559–563.

[6] Ding BS, Nolan DJ, Butler JM, James D, Babazadeh AO, Rosenwaks Z, et al. Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. Nature 2010;468:310–315.

[7] Xie G, Wang X, Wang L, Wang L, Atkinson RD, Kanel GC, et al. Role of differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic fibrosis in rats. Gastroenterology 2012;142:918–927.

[8] Wang L, Wang X, Xie G, Wang L, Hill CK, DeLeve LD. Liver sinusoidal endothelial cell progenitor cells promote liver regeneration in rats. J Clin Invest 2012;122:1567–1573.

[9] Narita M, Oussoultzoglou E, Chenard MP, Rosso E, Casnedi S, Pessaux P, et al. Sinusoidal obstruction syndrome compromises liver regeneration in patients undergoing two-stage hepatectomy with portal vein embolization. Surg Today 2011;41:7–17.

[10] Nakamura T, Torimura T, Sakamoto M, Hashimoto O, Taniguchi E, Inoue K, et al. Significance and therapeutic potential of endothelial progenitor cell transplantation in a cirrhotic liver rat model. Gastroenterology 2007;133:91–107.