Could endothelial TGF signaling be a promising new target for liver disease?

Adams, David H; Shepherd, Emma L; Lalor, Patricia F

DOI:
10.1080/17474124.2018.1477587

Document Version
Peer reviewed version

Citation for published version (Harvard):
Adams, DH, Shepherd, EL & Lalor, PF 2018, 'Could endothelial TGF signaling be a promising new target for liver disease?' Expert review of gastroenterology & hepatology, vol. 12, no. 7, pp. 637-639. https://doi.org/10.1080/17474124.2018.1477587

Editorial : Could endothelial TGFβ signalling be a promising new target for liver disease?

David H. Adams[1], Emma L. Shepherd[2] and Patricia F. Lalor[3]

[1]Professor of Hepatology and Dean of Medicine, [2]Research Fellow and [3]Senior Lecturer : National Institute for Health Research (NIHR) Birmingham Liver Biomedical Research Unit, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham B15 2TT United Kingdom.
p.f.lalor@bham.ac.uk

Keywords : endothelium, chronic liver disease, fibrosis, therapy, TGFB

Declaration : This editorial relates to independent research supported by the Birmingham NIHR Liver Biomedical Research Unit based at the University Hospitals Birmingham NHS Foundation Trust and the University of Birmingham. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Introduction

Chronic liver diseases are progressive and associated with increasing functional impairment as a consequence of organ fibrosis. Whilst much effort has been directed at therapies that address underlying causes of disease such as hepatitis viruses or metabolic impairment in non-alcoholic fatty liver disease (NAFLD), effective treatments to prevent or reverse fibrogenesis have proven elusive and many patients die from the systemic complications of their cirrhosis despite successful management of the initiating disease. Persistent injury to the liver parenchyma drives hepatic fibrosis as part of a dysregulated repair response. This is characterized by expansion of activated myofibroblasts which perpetuate the inflammation and increase production of extracellular matrix components such as collagen. Whilst many of the myofibroblasts arise as a consequence of activation of hepatic stellate cells, migration of cells from other sources such as the portal fibroblasts, bone marrow, and mesenchymal transformation of epithelial and endothelial cells all contribute to the myofibroblast pool[1]. Thus there is much interest in identifying factors that govern the activation of myofibroblasts or matrix remodeling that could be targeted in a therapeutic context[2]. The Transforming Growth Factor Beta (TGFβ) superfamily, consists of multiple individual protein isoforms and plays a key role in many fibrotic diseases. Dysregulated TGFβ signaling underpins virtually all fibrotic diseases, but the specific mechanisms and intracellular responses are context dependent. Here we consider a newly described contribution of TGFβ signaling in hepatic endothelial cells to consider whether this has potential as a therapy for fibrotic liver disease.

The multiple and complex roles of TGFβ

TGFβ is a pleiotropic cytokine with diverse roles in embryonic development, cell proliferation and differentiation, angiogenesis and wound healing responses. There are three protein isoforms TGFβ 1 to 3, which are similar structurally but vary in site of origin. These TGFβ isoforms play different roles in the scarring response, with TGFβ1 and β2 exerting pro-scarring effects and TGFβ3 favouring resolution. The TGFβ is initially secreted as a precursor form which undergoes conformational changes and proteolytic cleavage to generate a mature, active form and a latency associated peptide (LAP). There are various activators of TGFβ which include proteases that degrade LAP and molecules that non proteolytically disrupt the latent complex, including retinoids, MMPs and integrins[3, 4]. Activated TGFβ then binds to a receptor (TGFBR1 or 2) to initiate cellular responses. The downstream signaling initiated by TGFβ is complex and typically canonical (acting via Smad proteins) or non-canonical (Smad independent) leading to alterations in gene expression and function responses in target cells (reviewed in[5]). In the classical situation, activation of TGFBR2 phosphorylates TGFBR1 and Smad 2 and 3, these then interact with Smad 4, which can cross the nuclear membrane and modify target gene expression. Importantly co activators and repressors of this pathway such as Smad7 can regulate TGFβ responses in a cell and context specific manner.

Recently we collaborated with Anna Randi's group at Imperial College and have reported[6] that hepatic endothelial cell populations can contribute to the initiation of

fibrosis by undergoing endothelial to mesenchymal transition (EndoMT). Here endothelial cells lose the expression of characteristic cell surface markers and adopt a more mesenchymal phenotype in a process driven by TGFβ signaling. This process has been shown to contribute to fibrogenesis in atherosclerosis[7] where it is linked to disease severity, vascular rejection of organ transplants and fibrotic lung and heart[8] disease. The endothelial transcription factor ERG is a key regulator in this process, controlling the balance between profibrotic and homeostatic TGFβ signaling. The loss of ERG expression in response to proinflammatory signals in murine models of fibrosis and in a variety of human liver diseases including alcohol and fatty liver disease and cholangiopathies such as primary biliary cirrhosis (PBC), led to a smad3-dependent loss in endothelial ERG expression and induction of mesenchymal markers such as smooth muscle actin. This was associated with early periportal collagen deposition and could be reversed by using TNFα blockade to restore ERG expression and reduce smad3 activity, although there were disease-specific differences in the magnitude of the effect. This suggests that therapeutic interference with smad signaling or blockade of TNFα may serve to modify fibrosis in specific subgroups of patients, for example those with fibrosis arising from cholangiopathies, and also that components of the non-canonical TGFβ signaling pathway may represent useful prognostic indicators in patients with liver disease. Interestingly reports suggest that some patients with autoimmune hepatitis that is resistant to standard therapies show excellent responses to anti-TNFα therapy[9] supporting the potential of targeting TNF in hepatic fibrosis.

Are endothelial cells a good target for anti-fibrotic therapy?

The change in endothelial phenotype termed EndoMT is associated with an increasing number of pathological conditions including portal hypertension, diabetic vasculopathies and atherosclerosis. The endothelium becomes mesenchymal in nature with increased secretory, migratory and proliferative capabilities, and TGFβ has been implicated in driving the response in many situations. In atherosclerosis[7] TGFβ upregulation and signaling through Smad3 drives increased extracellular matrix deposition and smooth muscle actin expression in proportion to disease severity. This suggests that targeting endothelial signaling could control atheroma lesion development. This concept also holds true in diabetic nephropathy where EndoMT driven by TGFβ signaling activates endothelial Smad3 and contributes to local collagen deposition. Here therapeutic potential is supported by the ability of a Smad3 activation inhibitor SIS3 to reduce both EndoMT and renal fibrosis in a diabetic model[10]. However it important to note that TGFβ has varied effects in endothelial cells that depend on the context and timing of signaling. Thus signaling associated with Smad1/5 is vital for proliferation in angiogenesis whereas activation of Smad2/3 inhibits both proliferation and migration. Similarly, populations of endothelial cells respond differently to Smad activation in a spatiotemporal manner that is in part dependent on the hemodynamic shear stress they are exposed too. Previous studies have associated changes in liver sinusoidal endothelial cell function (LSEC) with the development of fibrosis in end stage liver disease. Hepatic sinusoidal endothelial cells change their phenotype in chronic disease with the loss of characteristic surface receptors and fenestrations and the development of a more

complex basement membrane in a process known as capillarization, analogous to EndoMT. This change precedes the development of fibrosis in a variety of liver diseases[12] and importantly maintenance of mature, differentiated LSEC function prevents stellate cell activation. The finding that [6] endothelial ERG expression is linked to TGF signalling and periportal fibrosis adds credence to the concept of vascular targeting as a tool to modify fibrogenesis in the liver. However, an important caveat relates to the possibility that different subpopulations of hepatic endothelial cells (eg portal *vs* sinusoidal or lymphatic) may respond or contribute differently in the context of specific liver diseases.

Is targeting TGFβ signaling specifically a good strategy?

An endothelial cell contribution to fibrogenesis has been reported in situations such as atheroma and nephropathy where the extent or proportion of fibrosis is relatively modest compared to that seen in hepatic cirrhosis. In the context of liver fibrosis, the proportion of scar tissue derived from dedifferentiated endothelial cells is likely to be small compared to that from the stellate-derived myofibroblasts. However, because reciprocal signaling between endothelial cells and hepatic stellate cells maintains both in a mature quiescent state, targeting the endothelium may also indirectly inhibit myofibroblast activation. In addition, although reducing the generation of extracellular matrix is of potential therapeutic value, reversal of cirrhosis requires extensive remodeling of established fibrotic tissue in an environment where the cytokine and growth factor milieu favours matrix crosslinking and stabilization rather than retraction resulting in scar tissue resistant to fibrolysis. It is likely that anti-fibrotic therapies will need to address the multi-factorial aspect of fibrosis including reducing inflammation, preventing further deposition of scar, enhancing breakdown of pre-existing scar as well as promoting regeneration of normal liver architecture. Alteration in endothelial TGFβ signaling alone is unlikely to accomplish this. Furthermore, there is a requirement to regenerate the damaged liver, and inhibition of TGFβ leads to morphological abnormalities, lack of endothelialization and stromal paucity in other tissues[13]. TGFβ -deficient mice die *in utero* due to a lack of normal vessel development and impaired angiogenesis is a feature of endothelial cells lacking the TGFβ receptor endoglin[14]. Furthermore endothelial TGFβ is central to the local control of VEGF production and hepatocyte regeneration [15]. Thus targeting endothelial TGFβ signaling could have unwanted effects on angiogenesis and hepatic regeneration. Endothelial cells can also present TGFβ to activate and maintain local regulatory T cells that are vital for control of immune responses and the prevention of autoimmunity[16]. Thus blockade of TGF responses could have detrimental effects on local immune regulation and thereby exacerbate chronic inflammation.

In conclusion, there may be specific clinical circumstances where targeting EndoMT or angiogenic responses by modifying endothelial TGFβ signaling may be of benefit. One possibility could be to target angiogenesis in hepatic tumours, as it is clear that TGFβ1 is particularly relevant in this context and as most primary liver tumours arise in the context of cirrhosis, effects on fibrosis could also be investigated. However, there is a need to better understand the complex effects of TGFβ and to develop methods specifically directed at the vasculature to minimize off target effects.

Therapies can be directed specifically to hepatic endothelium by targeting receptors such as aminopeptidase P, and PV-1 which are restricted to endothelium with caveoli, or by targeting the range of scavenger receptors expressed on sinusoidal but not vascular endothelium. In the context of liver fibrosis understanding the precise contribution of endothelial TGFβ contribution to the development of fibrogenesis is critical to determine whether therapeutic strategies are likely to be effective and if so when, and with what other treatments they should be combined.

References

1 Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut. 2015 May;64: 830-41.

2 Rosenbloom J, Mendoza FA, Jimenez SA. Strategies for anti-fibrotic therapies. Biochim Biophys Acta. 2013 Jul;1832: 1088-103.

3 Kojima S, Rifkin DB. Mechanism of retinoid-induced activation of latent transforming growth factor-beta in bovine endothelial cells. J Cell Physiol. 1993 May;155: 323-32.

4 Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev. 2000 Jan 15;14: 163-76.

5 Achyut BR, Yang L. Transforming growth factor-beta in the gastrointestinal and hepatic tumor microenvironment. Gastroenterology. 2011 Oct;141: 1167-78.

6 Dufton NP, Peghaire CR, Osuna-Almagro L, et al. Dynamic regulation of canonical TGFbeta signalling by endothelial transcription factor ERG protects from liver fibrogenesis. Nat Commun. 2017 Oct 12;8: 895.

**** The authours describe the EndMT process in human liver disease and highlight that ERG may be a novel endothelial cell target to TGFβ regulation in liver disease.**

7 Chen PY, Qin L, Baeyens N, et al. Endothelial-to-mesenchymal transition drives atherosclerosis progression. J Clin Invest. 2015 Oct 26;125: 4514-28.

8 Piera-Velazquez S, Mendoza FA, Jimenez SA. Endothelial to Mesenchymal Transition (EndoMT) in the Pathogenesis of Human Fibrotic Diseases. J Clin Med. 2016 Apr 11;5.

*** This review summarises the contribution of the EndoMT process to a number of human fibrotic disease pathologies.**

9 Weiler-Normann C, Schramm C, Quaas A, et al. Infliximab as a rescue treatment in difficult-to-treat autoimmune hepatitis. J Hepatol. 2013 Mar;58: 529-34.

10 Li J, Qu X, Yao J, et al. Blockade of endothelial-mesenchymal transition by a Smad3 inhibitor delays the early development of streptozotocin-induced diabetic nephropathy. Diabetes. 2010 Oct;59: 2612-24.

11 Beets K, Staring MW, Criem N, et al. BMP-SMAD signalling output is highly regionalized in cardiovascular and lymphatic endothelial networks. BMC Dev Biol. 2016 Oct 10;16: 34.

12 DeLeve LD. Liver sinusoidal endothelial cells in hepatic fibrosis. Hepatology. 2015 May;61: 1740-6.

13 Saika S, Saika S, Liu CY, et al. TGFbeta2 in corneal morphogenesis during mouse embryonic development. Dev Biol. 2001 Dec 15;240: 419-32.
14 Park S, Dimaio TA, Liu W, Wang S, Sorenson CM, Sheibani N. Endoglin regulates the activation and quiescence of endothelium by participating in canonical and non-canonical TGF-beta signaling pathways. J Cell Sci. 2013 Mar 15;126: 1392-405.
15 Fabregat I, Moreno-Caceres J, Sanchez A, et al. TGF-beta signalling and liver disease. Febs J. 2016 Jun;283: 2219-32.
*** In depth review of the roles of TGFβ signalling in liver disease pathogenesis.**
16 Carambia A, Freund B, Schwinge D, et al. TGF-beta-dependent induction of CD4CD25Foxp3 Tregs by liver sinusoidal endothelial cells. J Hepatol. 2014 May 2.