Endoglin in liver fibrosis

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Abstract Liver fibrosis occurs in most types of chronic liver diseases and is characterized by excessive accumulation of extracellular matrix proteins, leading to disruption of tissue function and eventually organ failure. Transforming growth factor (TGF)- β represents an important pro-fibrogenic factor and aberrant TGF- β action has been implicated in many disease processes of the liver. Endoglin is a TGF- β co-receptor expressed mainly in endothelial cells that has been shown to differentially regulate TGF- β signal transduction by inhibiting ALK5-Smad2/3 signalling and augmenting ALK1-Smad1/5 signalling. Recent reports demonstrating upregulation of endoglin expression in pro-fibrogenic cell types such as scleroderma fibroblasts and hepatic stellate cells have led to studies exploring the potential involvement of this TGF- β co-receptor in organ fibrosis. A recent article by Meurer and colleagues now shows that endoglin expression is increased in transdifferentiating hepatic stellate cells in vitro and in two different models (carbon tetrachloride intoxication and bile duct ligation) of liver fibrosis in vivo. Moreover, they show that endoglin overexpression in hepatic stellate cells is associated with enhanced TGF- β -driven Smad1/5 phosphorylation and α -smooth muscle actin production without altering Smad2/3 signaling. These findings suggest that endoglin may play an important role in hepatic fibrosis by altering the balance of TGF- β signaling via the ALK1-

Smad1/5 and ALK-Smad2/3 pathways and raise the possibility that targeting endoglin expression in transdifferentiating hepatic stellate cells may represent a novel therapeutic strategy for the treatment of liver fibrosis.

Keywords Endoglin, Liver fibrosis, TGF- β signaling · TGF- β co-receptor, Hepatic stellate cells

Transforming growth factor (TGF)- β is a multifunctional cytokine that plays critical roles in development and homeostasis by regulating cell proliferation, differentiation and extracellular matrix (ECM) production (Santibanez et al. 2011). Given that TGF- β is one of the most potent profibrotic cytokines known, it is not surprising that excessive TGF- β signalling has been implicated in a number of fibrotic conditions (Biernacka et al. 2011). Canonical TGF- β signalling occurs by engaging a pair of transmembrane serine-threonine receptors known as the type I (also known as activin-like receptor kinase-5 or ALK-5) and type II TGF- β receptors. Binding of TGF- β to the type II receptor leads to the trans-phosphorylation and activation of ALK5 which then conveys the signal by phosphorylating intracellular Smad2 and Smad3 (Massague and Gomis 2006). The activated Smad2/3 in turn form a complex with Smad4 and enter the nucleus where they interact with cell-type specific co-activators or co-repressors to regulate gene expression (Schmierer and Hill 2007). Recent evidence indicates that, in certain cell types, TGF- β also signals through another type I receptor, ALK1, resulting in the activation of Smad1/5 and a different transcriptional outcome (Daly et al. 2008; Finnon et al. 2008; Goumans et al. 2003). In these cell types where both the TGF- β /ALK5 and TGF- β /ALK1 pathways are active, the ALK1 pathway opposes the ALK5 pathway through mechanisms not yet fully delineated (Finnson et al. 2010; Lebrin et al. 2004; Morris et al. 2011).

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Endoglin (CD105) is a TGF- β co-receptor that regulates TGF- β signalling activity. Endoglin binds TGF- β 1 and TGF- β 3 isoforms in the presence of the type II receptor and regulates ligand affinity of the signalling receptors (Bernabeu et al. 2009). In addition, endoglin interacts with and is phosphorylated by the type II receptor, ALK5 and ALK1, and in turn has been shown to alter the phosphorylation status of the type II receptor and ALK5 (Guerrero-Esteo et al. 2002; Koleva et al. 2006; Ray et al. 2010). The recent findings that endoglin inhibits the ALK5-Smad2/3 pathway while promoting activation of the ALK1-Smad1/5 pathway, and thus acts as a critical factor in regulating the balance of signalling between these two pathways, provide further insight into the mechanism by which endoglin may regulate TGF- β signalling (Finnson et al. 2010; Lebrin et al. 2004; Morris et al. 2011). Mutations in endoglin or ALK1 have been shown to cause the vascular disorder, hereditary hemorrhagic telangiectasia (HHT-1 and HHT-2 respectively), suggesting that both proteins function in the same pathway (Bernabeu et al. 2010). There are 2 protein variants of endoglin, arising by alternative splicing. L-endoglin, the most common form, has 47 residues in its cytoplasmic domain while the shorter S-endoglin has a cytoplasmic tail of only 14 amino acids (Bellon et al. 1993). Also, the extracellular domain of endoglin can give rise to soluble endoglin by proteolytic cleavage (shedding) (Hawinkels et al. 2010).

Although previously thought to be expressed predominantly in endothelial cells, current evidence indicates that endoglin is expressed in other cell types including epithelial and mesenchymal cells (Morris et al. 2011; Quintanilla et al. 2003) suggesting that its role in regulating TGF- β signalling extends beyond the vascular system. Recent studies demonstrating endoglin expression in profibrogenic cells such as mesangial cells, scleroderma fibroblasts and hepatic stellate cells, have sparked an interest in understanding the potential role of this versatile TGF- β co-receptor in organ fibrosis. In the rat kidney, endoglin expression was shown to be increased following ureteral obstruction (Prieto et al. 2005) and renal mass reduction (Rodriguez-Pena et al. 2001). In addition, several murine models of experimental renal fibrosis including adriamycin-induced nephropathy (Sadlier et al. 2004), unilateral ureteral obstruction (Rodriguez-Pena et al. 2002) and radiation-induced fibrosis (Kruse et al. 2009) were shown to display enhanced endoglin expression in the kidney. Furthermore, endoglin haploinsufficiency was associated with reduced radiation-induced renal fibrosis in mice (Scharpfenecker et al. 2009) suggesting that endoglin may play a causative role in the pathogenesis of renal fibrosis. In addition, studies showing that increased endoglin expression may mediate angiotensin II-induced profibrotic responses in rat cardiac fibroblasts in vitro (Chen et al. 2004) and that decreased endoglin expression at least in

part may account for the anti-fibrotic effects of atorvastatin in a rat model of volume overload heart failure in vivo (Shyu et al. 2010), implicate endoglin in cardiac fibrosis.

Emerging evidence now indicates that endoglin may also play a pivotal role in liver fibrosis. The central role of TGF- β in chronic liver diseases is well documented (Dooley and Ten Dijke 2011). TGF- β contributes to all stages of liver disease progression ranging from the early events in injury and inflammation to the later processes of fibrosis/cirrhosis and hepatocellular carcinoma (Dooley and Ten Dijke 2011). Endoglin has been shown to be expressed in hepatic stellate cells (Meurer et al. 2005), which are the most fibrogenic cell type in the liver. Several studies have reported increased levels of soluble endoglin in the circulation during liver fibrosis, leading to the suggestion that increased expression of endoglin and subsequent release of its ectodomain from liver cells may occur during liver fibrosis (Clemente et al. 2006; Preativatanyou et al. 2010; Yagmur et al. 2007). A recent study by Meurer and colleagues (Meurer et al. 2011) now shows that endoglin expression is increased in transdifferentiating hepatic stellate cells in vitro and in two different models (carbon tetrachloride intoxication and bile duct ligation) of liver fibrosis in vivo, raising the possibility that endoglin may represent a biomarker for the progression of fibrosis in liver disease and/or a molecular target for the treatment of liver fibrosis.

Meurer and co-workers show that endoglin interacts with and is phosphorylated by the type II TGF- β receptor and that endoglin expression is upregulated by TGF- β in hepatic stellate cells. Furthermore, they demonstrate that transient overexpression of endoglin in a hepatic stellate cell line leads to a strong increase in TGF- β 1-driven Smad1/5 phosphorylation and α -smooth muscle actin expression while Smad2 phosphorylation is not altered. These results are in agreement with previous reports demonstrating that endoglin promotes TGF- β 1/ALK1-Smad1/5 signalling in endothelial cells (Lebrin et al. 2004) and chondrocytes (Finnson et al. 2010). Further functional studies in quiescent and transdifferentiating HSCs using siRNA knockdown of endogenous endoglin and measurement of basal and TGF- β -induced Smad2/3 versus Smad1/5 phosphorylation and the levels of ECM components (type I collagen, and fibronectin and α -smooth muscle actin) and related factors implicated in fibrosis (CTGF and ET-1) will provide important insight into the pro- or anti-fibrotic actions of endoglin. It will also be important to determine whether altering the expression of endoglin in transdifferentiating hepatic stellate cells in vitro and in the two in vivo models of liver fibrosis is associated with a corresponding change in the Smad1/5 phosphorylation. Such studies are needed to confirm that endoglin enhances TGF- β 1-mediated Smad1/5 activation leading to liver fibrosis.

Meurer et al. in the same study also report the identification of a novel form of L-endoglin designated as S'-endoglin that contains an in-frame intronic sequence which encodes an additional 49 amino acids in the C-terminus. The authors demonstrate that rat L-endoglin and S'-endoglin share similar functional properties including the ability to be phosphorylated by the type II receptor and to promote TGF- β 1-induced Smad1/5 phosphorylation and α -SMA expression in a hepatic stellate cell line. However, the authors fail to identify which of the endoglin isoforms display increased expression in their in vitro and in vivo models, since the antibody used for immunoblotting detects both rat L- and S'-endoglin isoforms, and the RT-PCR results shown do not specify the isoform. Further work is needed to establish whether L-endoglin or S'-endoglin, or both, are increased during hepatic stellate cell transdifferentiation in vitro and in the two models of liver fibrosis in vivo. It is possible that the two isoforms may have distinct functions in vivo in light of a previous report showing that the human L-endoglin and S-endoglin isoforms differentially regulate TGF- β /Smad signalling with L-endoglin promoting ALK1-Smad1/5 signalling and S-endoglin promoting the ALK5-Smad2/3 signalling in rat myofibroblasts (Velasco et al. 2008).

The demonstration by Meurer and co-workers of the presence of the endoglin ectodomain in conditioned media of transdifferentiating hepatic stellate cells raises several important questions regarding the mode of endoglin action in liver fibrosis. For example, does the shedding of endoglin ectodomain by the hepatic stellate cells contribute to increased circulating levels of soluble endoglin during hepatic fibrosis? Do the membrane-tethered and soluble forms of endoglin play distinct roles in regulating the balance of TGF- β signalling via the ALK5-Smad2/3 versus ALK1-Smad1/5 pathways, or in controlling liver fibrosis? What is the nature of the protease(s) responsible for endoglin ectodomain shedding and how is their proteolytic activity regulated? Answers to these questions will undoubtedly provide important insight into the mechanisms by which endoglin regulates TGF- β action leading to fibrosis in the liver.

A recent study by another group reported a similar increase in endoglin expression in the liver following bile duct ligation in rats, but transfection of human endoglin did not alter liver fibrosis (Pérez-Barriocanal et al. 2011). These findings suggest that generalized overexpression of endoglin in the liver may not alter the fibrotic process in the liver. The broad spectrum of TGF- β effects in the liver are cell-type and disease-stage specific and therefore may preclude the use of a generalized alteration of endoglin expression. Thus targeting endoglin expression to the relevant cell type (hepatic stellate cells?) at the appropriate stage/time during the fibrotic process is likely to be critical to obtain the desired therapeutic outcome.

Hepatic fibrosis occurs in most types of chronic liver diseases and treatment of these conditions represents a major clinical challenge. Increased TGF- β levels induced by liver damage stimulate hepatic stellate cell activation and myofibroblast formation leading to increased ECM production and fibrosis. Because there are currently no therapies available that stop or slow the progression of liver fibrosis, there is a major unmet need for the discovery of new molecules which may serve as new targets for therapeutic intervention. The findings by Meurer et al. suggest that endoglin, a TGF- β co-receptor, might represent such a molecule and opens up possibilities for pre-clinical studies to test whether altering endoglin expression in hepatic stellate cells at the right time during the fibrotic process might influence the progression of hepatic fibrosis. Results obtained from such studies will also have relevance to other fibrotic conditions that display elevated endoglin expression such as scleroderma (Leask et al. 2002) and glomerulonephritis (Roy-Chaudhury et al. 1997).

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