It has to be the αv: myofibroblast integrins activate latent TGF-β1

Boris Hinz

Cell-mediated activation of latent TGF-β1 is a key promoting event in fibrosis in all organs. A new study shows that specific targeting of the αv subunit of integrins in fibrogenic myofibroblasts effectively reduces developing and established fibrosis in liver, kidneys and lungs (pages 1617–1624).

It is well established that TGF-β1 and myofibroblasts have central roles in the development of fibrosis; however, no effective therapy exists to date to treat this group of severe connective tissue disorders. Fibrosis is the pathological accumulation and stiffening of collagenous extracellular matrix and has devastating effects on organ function[1]. Often starting as a beneficial physiological repair response to organ injury with hemostatic, inflammatory and remodeling phases, fibrosis is characterized by the persistent activity of matrix-remodeling myofibroblasts[2]. Myofibroblasts differentiate from various precursor cells that differ according to the nature of the insult and the affected organ[2]. The binding of active TGF-β1 to high-affinity TGF-β1 receptors in the plasma membrane of these precursors induces TGF-β1 signaling, which generates contractile (remodeling) cell features by promoting α-smooth muscle actin (α-SMA) neoexpression and secretory cell functions such as collagen production[2].

TGF-β1, being the master control cytokine, and myofibroblasts, being the main cellular effectors, have been identified as prime targets for antifibrosis strategies[2], but targeting specificity remains a problem for both. Although TGF-β1 and TGF-β1 receptor antagonists inhibit myofibroblast activation in cell culture and suppress induced fibrosis of skin, lung, kidney and liver in animal models, these strategies bear the risk of adverse effects on inflammatory cells and epithelium that are growth regulated by TGF-β1 (refs. 1,3). Similarly, myofibroblasts do not seem to have unique markers or features to target, possibly because of their heterogeneous origins; coexpression of α-SMA and collagen type I is currently the most reliable way to identify and trace myofibroblasts independent of their origin[2,4], yet vascular smooth muscle cells, pericytes, bone marrow stromal cells and myoepithelial cells also express α-SMA, and fibroblasts are collagen I positive[2].

In this issue, Henderson *et al.*[5] hit two birds with one stone with a new strategy to specifically target TGF-β1–mediated differentiation of myofibroblasts using a platelet-derived growth factor receptor β (PDGFRβ)-Cre mouse model (Pdgfrb-Cre), as induction of PDGFRβ occurs early during myofibroblast differentiation from pericytes. Using the Pdgfrb-Cre model, the authors successfully deleted an activator of latent TGF-β1, the αv integrin subunit, leading to suppression of carbon tetrachloride–induced fibrosis in the liver, where hepatic stellate cells (HSCs) are the local pericyte population and the major source of myofibroblasts. HSCs lacking the αv integrin subunit and normal HSCs treated with αv integrin inhibitors were unable to activate latent TGF-β1 in culture and had reduced expression of profibrotic genes. Supply of active TGF-β1 to the culture rescued HSC fibrogenesis. Notably, pericyte-specific deletion of αv integrin also prevented bleomycin-induced lung and ureteric obstruction-induced kidney fibrosis in mice. Moreover, inhibition of all αv integrins with a small-molecule inhibitor effectively suppressed fibrosis in lung and kidney and even reversed liver fibrosis in mouse models. The work further shows that multiple αv integrins, expressed on pericyte-like cells, are collectively—not individually—required for the development of organ fibrosis by activating latent TGF-β1. Moreover, PDGFRβ expression is established as a selective feature of HSCs, and impeding myofibroblast differentiation from HSCs in the liver and from pericytes in lung and kidney suppresses fibrosis by eliminating a major fraction of profibrotic myofibroblasts (**Fig. 1**).

For the first time, Henderson *et al.*[5] have shown that PDGFRβ is also expressed in quiescent HSCs in the normal liver and serves as a marker for HSCs and their myofibroblast progenies. Perivascular pericytes also express PDGFRβ but do not seem to contribute substantially to the myofibroblast population in liver fibrosis. In an independent parallel study, Mederacke *et al.*[6] used a lecithin-retinol acyltransferase-driven Cre fluorescent reporter construct to fate-trace HSCs in the normal liver and in four different mouse models of induced liver fibrosis. Together, both studies indicate that HSCs are in fact the only numerically relevant precursors of myofibroblasts in the fibrotic liver (**Fig. 1**).

The antifibrosis strategy to eliminate αv integrins was motivated by previous findings showing that TGF-β1 is secreted in a latent form and stored in the ECM and that release of the active cytokine depends on the binding of the transmembrane integrins αvβ1, αvβ3, αvβ5, αvβ6 and αvβ8 to an arginine-glycine–aspartic acid (RGD) consensus sequence in the latent TGF-β1 complex[7]. In mouse lungs, deletion or blocking of the epithelial integrin αvβ6 alone is sufficient to prevent latent TGF-β1 activation and development of bleomycin-induced fibrosis without inducing the side effects of global TGF-β1 inhibition[8,9]. Yet, the liver of αvβ6-deficient mice is not protected from fibrosis. It is further difficult to imagine how blocking an epithelium-specific integrin would prevent fibrosis in the heart or muscle, which lack epithelia. In these conditions, mesenchymal cells come into play, expressing and upregulating all of the remaining αv integrins

Boris Hinz is at the Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Toronto, Canada.
e-mail: boris.hinz@utoronto.ca

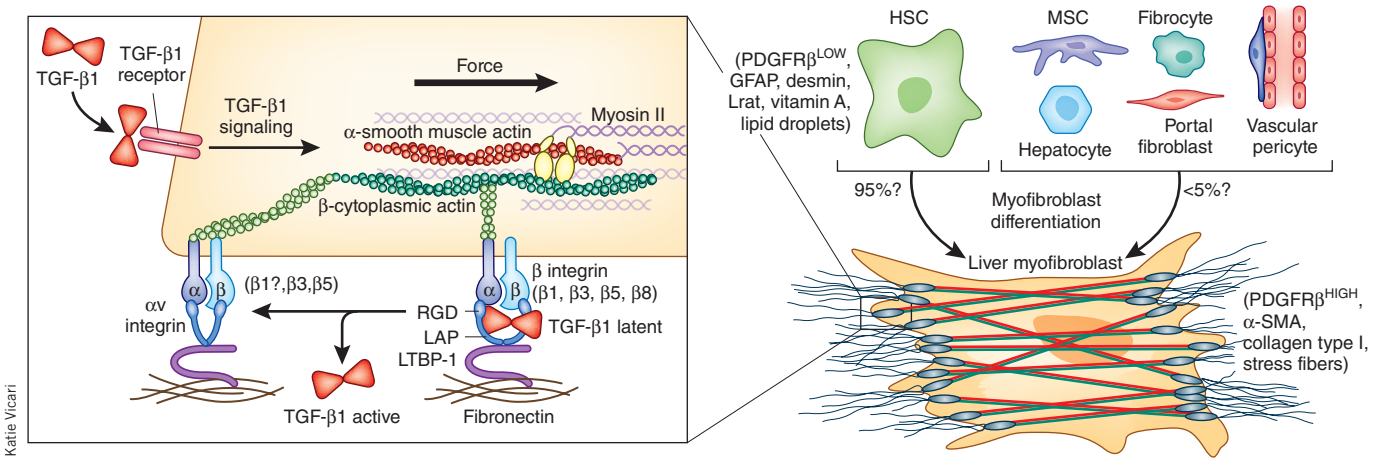

Figure 1 Latent TGF-β1 activation by αv integrins contribute to myofibroblast differentiation in hepatic stellate cells. To date, PDGFRβ and lecithin-retinol acyltransferase (Lrat) are the most specific marker proteins to identify and fate-trace HSCs. In different mouse models of induced liver fibrosis, activation of HSCs supplies the vast majority of fibrogenic myofibroblasts, and other cells seem to have a lesser role. Liver myofibroblasts promote fibrosis by secreting excessive amounts of collagen and developing high contractile force by virtue of α-SMA neoexpression in stress fibers, which are promoted upon binding of profibrotic active TGF-β1 to its receptor. Henderson *et al.*[5] showed that activation of latent TGF-β1 from the extracellular matrix by αv integrins is a key event in HSC-to-myofibroblast differentiation and that inhibition or deletion of the αv subunit blocks liberation of active TGF-β1, myofibroblast differentiation and development of liver fibrosis. Although αvβ3, αvβ5 and αvβ8 seem to be compensatory in their function of activating latent TGF-β1, αvβ1 integrin could be also the major integrin to activate latent TGF-β1. GFAP, glial fibrillary acidic protein; LAP, latency-associated peptide; LTBP-1, latent TGF-β1 binding protein.

during myofibroblast differentiation in conditions of organ fibrosis. Surprisingly, global deletion of individual β integrin subunits that only pair with αv integrin (such as β3, β5 and β8) does not protect against liver fibrosis[5].

Two explanations are possible: First, mesenchymal cells are opportunistic and use whatever integrins are available to activate latent TGF-β1. Rapid tissue repair by TGF-β1–differentiated myofibroblasts is fundamental for organism survival, and it is conceivable that different αv integrins are redundant in their function of latent TGF-β1 activation. One supporting fact for this idea is that deletion of all HSC-specific αv integrins[5] and of the epithelial integrin αvβ6 (ref. 8) similarly protect from lung fibrosis. Although epithelial cells express β6 integrin as the only partner for the αv integrin, mesenchymal cells can compensate for the loss of any β integrin by pairing αv with alternative β integrin subunits. Indeed, different αv integrins have been shown to activate latent TGF-β1 *in vitro* either by supporting proteolytic activation, for example, integrin αvβ8 (ref. 10), or by inducing a conformational change in latent TGF-β1 through cytoskeletal force transmission[11,12]. It is conceivable that different αv integrins contribute to latent TGF-β1 activation in a spatiotemporal hierarchy. For instance, αvβ6 integrin may be more important for the onset of lung fibrosis upon lung epithelial injury, whereas 'mesenchymal' αv integrins drive the progression and persistence of the disease, distant from the original insult.

Another possible explanation for the failure of β3, β5 and β8 single deletions to protect against liver fibrosis is that integrins αvβ3, αvβ5 and αvβ8 have no physiological role in hepatic latent TGF-β1 activation. In this case, the only remaining αv integrin that binds or activates latent TGF-β1would be αvβ1, whose function is still enigmatic. No reagents or antibodies exist to detect αvβ1 integrin, and a specific deletion is not possible as αv and β1 integrin subunits both pair with multiple partners. After this 'forgotten integrin' was discovered in the early 1990s as a fibronectin receptor[13], it stopped making news; however, the study by Henderson *et al.*[5] revived interest in the earlier detection of αv and β1 integrin in coimmunoprecpitates using latent TGF-β1 as a ligand[14]. Hence, αvβ1 integrin is possibly part of the group of latent TGF-β1–activating integrins in pericytes and may even turn out to be the leader of the pack.

The ultimate question remains: what are the possible side effects of a pharmaceutical anti-αv integrin therapy? Smooth muscle cells, pericytes and endothelial cells express integrins αvβ3 and αvβ5, making them attractive targets to prevent tumor growth by blocking neovascularization[15]. In the reported mouse models of liver, lung and kidney fibrosis, blocking of αv integrin with a peptide inhibitor did not show any adverse effects on HSC adhesion and migration, vascular pericyte numbers or neovascularization upon fibrosis[5]. Hence, we can still hope for a magic bullet directed against fibrosis with minimal adverse effects.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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