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It has to be the α v: myofibroblast integrins activate latent TGF- β 1

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Cell-mediated activation of latent TGF- β 1 is a key promoting event in fibrosis in all organs. A new study shows that specific targeting of the α v subunit of integrins in fibrogenic myofibroblasts effectively reduces developing and established fibrosis in liver, kidneys, and lungs.

It is well established that TGF- β 1 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 with devastating effects on organ function¹. 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 remodelling myofibroblasts². Myofibroblasts differentiate from various precursor cells that differ according to the nature of the insult and the affected organ². The binding of active TGF- β 1 to high-affinity TGF- β 1 receptors in the plasma membrane of these precursors induces TGF- β 1 signalling, which generates contractile (remodelling) cell features by promoting α -smooth muscle actin (α -SMA) neo-expression and secretory cell features such as collagen production².

TGF- β 1, being the master control cytokine, and myofibroblasts, being the main cellular effectors, have been identified as prime targets for anti-fibrosis strategies², but targeting specificity remains a problem for both. Although TGF- β 1 and TGF- β 1 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- β 1^{1,3}. Similarly, myofibroblasts do not seem to possess unique markers or features to target, possibly because of their heterogeneous origins - co-expression 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 positive².

In this issue of *Nature Medicine*, Henderson *et al.*⁵ hit two birds with one stone with a new strategy to specifically target TGF- β 1-mediated differentiation of myofibroblasts using a platelet-derived growth factor receptor β (PDGFR β)-Cre mouse model (*Pdgfrb*-Cre), as induction of PDGFR β occurs early during myofibroblast differentiation from pericytes. Using the *Pdgfrb*-Cre, the authors successfully deleted an activator of latent TGF- β 1, the α v 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 α v integrin subunit and normal HSCs treated with α v integrin inhibitors were unable to activate latent TGF- β 1 in culture and had reduced expression levels of pro-fibrotic genes. Supply of active TGF- β 1 to the culture rescued HSC fibrogenesis. Notably, pericyte-specific deletion of α v integrin also prevented bleomycin-induced lung and ureteric obstruction-induced kidney fibrosis in mice. Moreover, inhibition of all α v integrins with a small molecule inhibitor effectively suppressed fibrosis in lung and kidney and even reverted liver fibrosis in mouse models. The work further shows that multiple α v integrins, expressed on pericyte-like cells, are collectively—not individually—required for the development of organ fibrosis by activating latent TGF- β 1. Moreover, PDGFR β 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 pro-fibrotic myofibroblasts (**Fig.1**).

For the first time, Henderson *et al.*⁵ have showed that PDGFR β 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 PDGFR β 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 anti-fibrosis strategy to eliminate αv integrins was motivated by previous findings showing that TGF- $\beta 1$ 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 $\alpha v \beta 1$, $\alpha v \beta 3$, $\alpha v \beta 5$, $\alpha v \beta 6$, and $\alpha v \beta 8$ to an arginine-glycine-aspartic acid (RGD) consensus sequence in the latent TGF- $\beta 1$ complex⁷. In mouse lungs, deletion or blocking of the epithelial integrin $\alpha v \beta 6$ alone is sufficient to prevent latent TGF- $\beta 1$ activation and development of bleomycin-induced fibrosis without inducing the side effects of global TGF- $\beta 1$ inhibition^{8,9}. Yet, the liver of $\alpha v \beta 6$ -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, lacking epithelia. In these conditions, mesenchymal cells come into play, expressing and upregulating all remaining αv integrins during myofibroblast differentiation in conditions of organ fibrosis. Surprisingly, global deletion of individual β integrin subunits that only pair with αv integrin (such as $\beta 3$, $\beta 5$, and $\beta 8$) does not protect against liver fibrosis⁵.

Two explanations are possible: First, mesenchymal cells are opportunistic and use whatever integrins available to activate latent TGF- $\beta 1$. Rapid tissue repair by TGF- $\beta 1$ -differentiated myofibroblasts is fundamental for organism survival and it is conceivable that different αv integrins are redundant in their function of latent TGF- $\beta 1$ activation. One supporting fact for this idea is that deletion of all HSC-specific αv integrins⁵ and of the epithelial integrin $\alpha v \beta 6$ ⁸ similarly protect from lung fibrosis. While epithelial cells express $\beta 6$ integrin as the only partner for the αv integrin, mesenchymal cells can compensate 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- $\beta 1$ *in vitro* either by supporting proteolytic activation, like integrin $\alpha v \beta 8$ ¹⁰, or by inducing a conformational change in latent TGF- $\beta 1$ through cytoskeletal force transmission^{11,12}. It is conceivable that different αv integrins contribute to latent TGF- $\beta 1$ activation in a spatio-temporal hierarchy. For instance,

$\alpha\text{v}\beta 6$ 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 $\beta 3$, $\beta 5$, and $\beta 8$ single-deletions to protect against liver fibrosis is that integrins $\alpha\text{v}\beta 3$, $\alpha\text{v}\beta 5$, and $\alpha\text{v}\beta 8$ play no physiological part in hepatic latent TGF- $\beta 1$ activation. In this case, the only remaining latent TGF- $\beta 1$ binding/activating αv integrin would be $\alpha\text{v}\beta 1$ whose function is still enigmatic. No reagents or antibodies exist to detect $\alpha\text{v}\beta 1$ integrin and a specific deletion is not possible as αv and $\beta 1$ integrin subunits both pair with multiple partners. After its discovery in the early 1990’s as a fibronectin receptor¹³, it became quiet around this ‘forgotten integrin’; however, the study by Henderson *et al.*⁵ revived the earlier detection of αv and $\beta 1$ integrin in co-immunoprecipitates using latent TGF- $\beta 1$ as a ligand¹⁴. Hence, $\alpha\text{v}\beta 1$ integrin is possibly part of the group of latent TGF- $\beta 1$ -activating integrins in pericytes and may even turn out to be the leader of the pack.

The ultimate question remains is what are the possible side effects of a pharmaceutical anti- αv integrin therapy? Smooth muscle cells, pericytes and endothelial express integrins $\alpha\text{v}\beta 3$ and $\alpha\text{v}\beta 5$ making them attractive targets to prevent tumor growth by blocking neo-vascularization¹⁵. 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 neo-vascularization upon fibrosis⁵. Hence, we can still hope for a magic bullet directed against fibrosis with minimal adverse effects.

Figure 1: Latent TGF- $\beta 1$ activation by αv integrins contribute to myofibroblast differentiation in hepatic stellate cells. To date, platelet-derived growth factor receptor β (PDGFR β) and lecithin-retinol acyltransferase (Lrat), are the most specific marker proteins to identify and fate-trace hepatic stellate cells (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 -smooth muscle actin (α -SMA) neo-

expression in stress fibers, which are promoted upon binding of pro-fibrotic active TGF- β 1 to its receptor. Henderson *et al.*⁵ showed that activation of latent TGF- β 1 from the extracellular matrix by α v integrins is a key event in HSC-to-myofibroblast differentiation and that inhibition or deletion of the α v subunit blocks liberation of active TGF- β 1, myofibroblast differentiation, and development of liver fibrosis. Although α v β 3, α v β 5, and α v β 8 seem to be compensatory in their function of activating latent TGF- β 1, α v β 1 integrin could be also the major integrin to activate latent TGF- β 1. GFAP, glial fibrillary acidic protein; LAP, Latency associated peptide; LTBP-1, latent TGF- β 1 binding protein.

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