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## Integrin-mediated regulation of TGF $\beta$ in fibrosis

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### Abstract

Fibrosis is a major cause of morbidity and mortality worldwide. Currently, therapeutic options for tissue fibrosis are severely limited, and organ transplantation is the only effective treatment for end-stage fibrotic disease. However, demand for donor organs greatly outstrips supply, and so effective anti-fibrotic treatments are urgently required. In recent years the integrin family of cell adhesion receptors have gained prominence as key regulators of chronic inflammation and fibrosis. Fibrosis models in multiple organs have demonstrated that integrins have profound effects on the fibrotic process. There is now abundant *in vivo* data demonstrating critical regulatory roles for integrins expressed on different cell types during tissue fibrogenesis. In this review we will examine the ways in which integrins regulate these processes and discuss how the manipulation of integrins using function blocking antibodies and small molecule inhibitors may have clinical utility in the treatment of patients with a broad range of fibrotic diseases.

### Keywords

Integrins; fibrosis; TGF $\beta$

### Introduction

Fibrosis represents a massive health care burden worldwide. Chronic tissue injury with fibrogenesis results in disruption of tissue architecture, organ dysfunction and eventually organ failure. Our therapeutic repertoire for the treatment of tissue fibrosis is severely limited and organ transplantation is currently the only effective treatment in end-stage fibrotic disease. However, organ transplantation has several disadvantages including limited donor organ availability, high cost, co-morbidities in potential recipients and on a global scale, organ transplantation can only be offered to a small percentage of the patients suffering from the complications of fibrosis. Therefore there is an urgent imperative to develop effective anti-fibrotic therapies.

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Conflict of interest statement: Dean Sheppard is a co-owner of patents covering targeting the  $\alpha$ v $\beta$ 6 integrin for treatment of pulmonary fibrosis

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A universal feature of tissue fibrogenesis is the complex interplay between the inflammatory, epithelial, myofibroblast and extracellular matrix components of the wound healing response<sup>1,2,3</sup>. Furthermore, the pericellular extracellular matrix is a highly dynamic environment known to exert profound influences on cell behaviour. Many of the key cell-cell and cell-matrix interactions which regulate fibrosis are mediated by members of the integrin family of cell adhesion molecules, of which there are 24 known members in humans (noncovalent  $\alpha/\beta$  heterodimers composed from 18 different  $\alpha$  subunits and 8  $\beta$  subunits). Integrins represent a major mode of communication between the extracellular matrix, inflammatory cells, fibroblasts and parenchymal cells and hence are intimately involved in the processes that govern the initiation, maintenance and resolution of tissue fibrosis. Integrins are transmembrane proteins and are major receptors for cell adhesion to extracellular matrix proteins and cell-cell adhesion<sup>4</sup>. These molecules can therefore mediate the translation of spatially fixed extracellular signals into a wide variety of changes in cell behavior including cell adhesion, migration, proliferation, differentiation and apoptosis<sup>4,5</sup>. In addition to their direct effects on cellular proliferation and survival, integrins can also potentiate signals from soluble growth and survival factors. For example, nearly all of the pro-fibrogenic cytokine transforming growth factor beta 1 (TGF $\beta$ 1) is secreted and bound to the extracellular matrix in a latent form, and therefore conversion to an active form is an important step in the regulation of TGF $\beta$ 1 activity. In recent years it has become clear that a subset of the integrin family ( $\alpha$ v integrins) play a key role in the activation of latent TGF $\beta$ 1. Specifically, the integrins  $\alpha$ v $\beta$ 3,  $\alpha$ v $\beta$ 5,  $\alpha$ v $\beta$ 6 and  $\alpha$ v $\beta$ 8 have been shown to bind the RGD sequence in the latency associated peptide (LAP) of TGF- $\beta$ 1 and - $\beta$ 3, and have the potential to activate latent TGF- $\beta$ <sup>6,7,8,9,10</sup>. In this review we will highlight recent data demonstrating the profound effects of integrins in modulating the fibrotic process via activation of TGF $\beta$ , and how pharmacologic manipulation of specific integrins may lead to the development of new antifibrotic treatments.

## Lung fibrosis

### $\alpha$ v integrin-mediated activation of latent TGF $\beta$

Secreted transforming growth factor beta 1 (TGF $\beta$ 1) is a major pro-fibrogenic cytokine and a key regulator of fibrosis in multiple organs<sup>11,12,13</sup>. Therefore, the molecular pathways that regulate TGF $\beta$ 1 activity and signaling are attractive targets for novel anti-fibrotic therapies. There are three mammalian isoforms of TGF $\beta$ , and all are synthesized as precursor proteins that are processed by proteolytic cleavage in the endoplasmic reticulum and assembled as a non-covalent complex of a disulfide linked homodimer of the mature cytokine (a short C-terminal fragment) and a disulfide linked homodimer of a larger amino terminal fragment called the latency associated peptide (LAP), forming the “small latent complex”. In this form the associated LAP homodimer prevents the mature C-terminal fragment from binding to its receptors and inducing TGF $\beta$ 's known effects. This “small latent complex” is further modified in the endoplasmic reticulum by disulfide linkage to another family of gene proteins called latent TGF $\beta$  binding proteins, which, upon secretion, are themselves chemically cross-linked to the extracellular matrix, to store and tether TGF $\beta$  in a latent form in the extracellular space. Much of the regulation of TGF $\beta$  biology thus occurs at the level of extracellular activation of this stored latent complex<sup>14,15</sup>.

Because the active form of TGF $\beta$  is non-covalently linked to the latency associated peptide and easily dissociates upon changes in temperature or pH<sup>15</sup>, *in vitro* examination of TGF $\beta$  activation has been difficult. Therefore, the *in vivo* mechanisms of matrix-bound latent TGF $\beta$  conversion into an active cytokine is a subject of intense research. Two of the three mammalian TGF $\beta$  isoforms (TGF $\beta$ 1 and 3) can be activated by members of the integrin family that interact with a linear arginine-glycine-aspartic acid (RGD) motif present in the latency associated peptide<sup>6,7,16</sup>. Inhibition and blockade of two of these integrins ( $\alpha$ v $\beta$ 6 and

$\alpha\nu\beta 8$ ) phenocopies all of the developmental effects of loss of TGF $\beta 1$  and 3<sup>17</sup>, suggesting that these two integrins are required for most or all important roles of these TGF $\beta$  isoforms during development. However, the mechanisms of TGF $\beta$  activation that contribute to tissue pathology in adults are less well understood.

In the lung, the  $\alpha\nu\beta 6$  integrin is minimally expressed in alveolar epithelial cells at baseline but is rapidly induced in this cell type following lung injury<sup>18</sup>. Evidence supporting an important role for the  $\alpha\nu\beta 6$  integrin in TGF $\beta 1$  activation came from observation of the phenotype of  $\beta 6$  integrin subunit knockout mice. These mice develop exaggerated inflammatory responses in the lungs and skin, reminiscent of, but less severe than the exaggerated inflammation seen in mice homozygous for a null mutation of TGF $\beta 1$ <sup>19</sup>. Furthermore, following treatment with bleomycin (a widely used inducer of pulmonary fibrosis),  $\beta 6$  null mice develop exaggerated inflammation but are dramatically protected from subsequent pulmonary fibrosis<sup>6</sup>.  $\beta 6$  inhibition (both by genetic knockout and blockade by anti- $\alpha\nu\beta 6$  antibodies) was also protective in radiation-induced pulmonary fibrosis<sup>20</sup>. The  $\alpha\nu\beta 6$  integrin can bind directly to the LAP of TGF $\beta 1$  and TGF $\beta 3$ <sup>16</sup> and cells expressing  $\alpha\nu\beta 6$  generate TGF $\beta 1$  activity *in vitro* that can be completely inhibited by  $\beta 6$  blocking antibodies. In addition, microarray analysis of the lungs of wild type or  $\beta 6$  null mice following intratracheal instillation of bleomycin identified a large group of TGF $\beta$ -inducible genes that were induced at substantially lower levels in  $\beta 6$  knockout mice<sup>21</sup>. Taken together, these data demonstrate that  $\alpha\nu\beta 6$  integrin expression on lung epithelial cells is a major regulator of TGF $\beta 1$  activation during lung fibrosis.

Activation of TGF $\beta 1$  was inhibited by blockade of actin polymerization<sup>6</sup> and by Rho kinase inhibition<sup>22</sup>, suggesting a role for force generation by the actin cytoskeleton. Indeed, the recently solved crystal structure of the small latent complex of TGF $\beta 1$  demonstrated that mechanical force generated by integrins is a common mechanism for activating latent TGF $\beta 1$ <sup>23</sup>. Shi and colleagues found that crystals of dimeric porcine proTGF- $\beta 1$  revealed a ring-shaped complex, a novel fold for the prodomain (LAP) of TGF $\beta 1$ , and demonstrated that the prodomain shields the growth factor from recognition by receptors and alters its conformation. Furthermore, complex formation between  $\alpha\nu\beta 6$  integrin and the prodomain of TGF $\beta 1$  was insufficient for TGF $\beta 1$  release, and force-dependent activation of TGF $\beta 1$  required unfastening of a “straitjacket” that encircles each growth factor monomer.

Myofibroblasts are a further cell type intrinsically involved in the fibrotic process, as they are the major source of extracellular matrix proteins during organ scarring. These contractile cells express several  $\alpha\nu$  integrins and force generated by the actomyosin cytoskeleton can be transmitted to the extracellular matrix by  $\alpha\nu$  integrins. Elegant *in vitro* studies of myofibroblasts have shown that these cells can utilize alternative  $\alpha\nu$  integrins to activate TGF $\beta 1$ , and demonstrates that myofibroblasts can liberate and activate TGF $\beta 1$  from pre-existing and self-generated deposits in the extracellular matrix by transmitting their high contractile force to the large latent complex through  $\alpha\nu\beta 5$  integrin and as yet unidentified  $\beta 1$  and 3 integrins<sup>10</sup>.

The integrin  $\alpha\nu\beta 8$  is also capable of binding to and activating TGF $\beta 1$ <sup>7</sup>. This was an unexpected finding, as  $\alpha\nu\beta 6$ -mediated activation was found to depend critically on sequences within the  $\beta 6$  cytoplasmic domain<sup>6</sup>, however the  $\beta 8$  cytoplasmic domain and the  $\beta 6$  cytoplasmic domain are completely divergent. In addition, even deletion of the  $\beta 8$  cytoplasmic domain did not diminish  $\alpha\nu\beta 8$ -mediated TGF $\beta 1$  activation, suggesting that these integrins (which both bind to the same RGD sequence in the TGF $\beta 1$  and TGF $\beta 3$  latency associated peptides) might activate the TGF $\beta 1$  latent complex by differing mechanisms. Further work demonstrated this to be the case. In contrast to  $\alpha\nu\beta 6$  mediated activation of TGF $\beta 1$ , which depends on direct cell-cell contact,  $\alpha\nu\beta 8$ -mediated activation

releases active TGF $\beta$ 1 into the culture medium of  $\alpha$ v $\beta$ 8 expressing cells. In addition, whereas  $\alpha$ v $\beta$ 6-mediated activation is completely resistant to inhibition by a variety of protease inhibitors, metalloprotease inhibitors abolish  $\alpha$ v $\beta$ 8-mediated TGF $\beta$ 1 activation, and transfection studies in cells demonstrated a role for the protease MT1-MMP (MMP14) in this process. Therefore  $\alpha$ v $\beta$ 8 appears to activate TGF $\beta$ 1 by presenting latent complexes to cell-surface metalloproteases which degrade the latency associated peptide and release free TGF $\beta$ 1 into the extracellular milieu. An important role for  $\alpha$ v $\beta$ 8-mediated TGF $\beta$ 1 activation *in vivo* is supported by studies of  $\beta$ 8 knockout mice. Some of these mice die in mid-gestation from a defect in vascular development reminiscent of that seen in some TGF $\beta$ 1 null mice<sup>24</sup>. Mice that survive to birth die soon after from brain haemorrhage that could be explained by loss of developmental vascular effects of TGF $\beta$ 1. Furthermore, many of these mice have a cleft palate, a prominent feature in TGF $\beta$ -3 knockout mice<sup>25</sup>.

These data strongly suggest that the  $\alpha$ v $\beta$ 8 integrin is an important regulator of TGF $\beta$ 1 and TGF $\beta$ 3 activation *in vivo*, but does manipulation of this integrin have any modulatory effect on the fibrotic process? Previous studies have shown that  $\alpha$ v $\beta$ 8 expression is increased in the airway fibroblasts of COPD (chronic obstructive pulmonary disease) patients and expression correlated with the extent of airway wall fibrosis. Furthermore,  $\alpha$ v $\beta$ 8-mediated activation of TGF $\beta$ 1 by COPD fibroblasts increased pro-fibrogenic differentiation<sup>26</sup>. Recently studies conducted by the same group have examined the role of fibroblast  $\alpha$ v $\beta$ 8 in murine airway fibrosis<sup>27</sup>. Kitamura et al. demonstrated that conditional deletion of lung fibroblast  $\alpha$ v $\beta$ 8 inhibited airway fibrosis in both IL-1 $\beta$  and ovalbumin-induced murine models of airway fibrosis. Furthermore, deletion of  $\alpha$ v $\beta$ 8 reduced TGF $\beta$ 1 activation by cultured mouse lung fibroblasts. Extending their studies to human lung fibroblasts, the authors also found that IL-1 $\beta$  enhanced  $\alpha$ v $\beta$ 8-dependent TGF $\beta$  activation, collagen expression and pro-inflammatory gene expression in COPD compared with normal lung fibroblasts.

### $\alpha$ 3 $\beta$ 1-mediated regulation of lung fibrosis

In recent years the origin of myofibroblasts in pulmonary fibrosis has been intensely studied, with potential sources including resident fibroblasts, circulating progenitors and epithelial-mesenchymal transition (EMT)<sup>28,29,30</sup>. Because the extracellular matrix is a key regulator of alveolar epithelial cell responses to TGF $\beta$ 1 (and this cytokine is a potent inducer of EMT *in vitro*), Kim and colleagues investigated the role of the prominent epithelial integrin  $\alpha$ 3 $\beta$ 1 (a laminin receptor known to co-localise with E-cadherin and  $\beta$ -catenin at adherens junctions<sup>31</sup>) in a mouse model of pulmonary fibrosis using mice with conditional epithelial cell-specific deletion of  $\alpha$ 3 integrin expression<sup>32</sup>. Despite a normal response to acute bleomycin-induced lung injury, these mice demonstrated a reduction in lung myofibroblasts and type I collagen and did not progress to fibrosis. To investigate whether this phenotype was secondary to a reduction in EMT, the authors examined  $\beta$ -catenin signalling as  $\beta$ -catenin has been implicated in EMT. They found that in primary alveolar epithelial cells  $\alpha$ 3 integrin was required for  $\beta$ -catenin phosphorylation at tyrosine residue 654 (Y654), formation of a pY654- $\beta$ -catenin/p-SMAD2 complex, and initiation of EMT both *in vitro* and *in vivo* during fibrosis following bleomycin-induced lung injury. Furthermore, analysis of human lung tissue from idiopathic pulmonary fibrosis (IPF) patients demonstrated pY654- $\beta$ -catenin-pSMAD2 complexes and accumulation of pY654- $\beta$ -catenin in myofibroblasts. This suggests that alveolar epithelial integrin-dependent crosstalk between  $\beta$ -catenin and Smad signaling is important during the evolution of lung fibrosis, and that EMT plays a role in the development of lung fibrosis. However, it should also be noted that a number of recent cell fate mapping studies in multiple organs including the lung, have shown that EMT does not directly contribute to the pool of collagen-producing myofibroblasts during fibrogenesis *in*

*in vivo*<sup>33,34,35</sup>. The molecular mechanisms of integrin-mediated regulation of lung fibrosis are summarized in Figure 1.

## Liver fibrosis

Integrin  $\alpha v \beta 6$  mRNA expression is increased in patients with fibrotic liver disease secondary to a variety of aetiologies (primary biliary cirrhosis, alcohol-induced, hepatitis B and C) and expression increases with fibrosis stage in hepatitis C<sup>36</sup>. Furthermore  $\alpha v \beta 6$  expression is virtually absent in normal liver but is significantly upregulated in rodent models of liver fibrosis<sup>36,37</sup>. Using the bile duct ligation model of acute biliary fibrosis Wang et al.<sup>37</sup> demonstrated that bile duct obstruction induces a marked increase in cholangiocyte  $\alpha v \beta 6$  expression. Furthermore, biliary fibrosis is reduced by 50% in  $\beta 6$  integrin null mice compared to wild type controls, and administration of a blocking antibody to  $\alpha v \beta 6$  significantly decreased acute fibrosis after bile duct ligation. A recent study has also examined the effect of a small molecule inhibitor of  $\alpha v \beta 6$  (EMD527040) during biliary fibrosis<sup>38</sup>. Biliary fibrosis was studied in rats after bile duct ligation and in  $Mdr2(abcb4)^{-/-}$  mice. Differing doses of EMD527040 were given to rats from week 2 to 6 after BDL and to  $Mdr2(abcb4)^{-/-}$  mice from weeks 4 to 8. EMD527040 reduced bile duct proliferation and peribiliary collagen deposition by 40–50%, decreased pro-fibrotic gene expression and up-regulated fibrolytic genes.

Hepatic stellate cells (Ito cells, liver specific pericytes) are the major source of extracellular matrix proteins during hepatic fibrogenesis<sup>39,40</sup>, and therefore represent an important target in the development of anti-fibrotic therapies for liver fibrosis. Zhou et al. examined the possibility that stellate cell fate is influenced by the extracellular matrix through the intermediary of  $\alpha v \beta 3$  integrin<sup>41</sup>.  $\alpha v \beta 3$  was expressed by rat and human culture-activated liver myofibroblasts, and blockade of this integrin inhibited stellate cell proliferation and increased apoptosis of cultured stellate cells. A recent study using cilengitide (an antagonist mainly selective for  $\alpha v \beta 3$  and  $\alpha v \beta 5$ , with less potency towards  $\alpha v \beta 6$ ) demonstrated a 30% increase in hepatic collagen in two models of liver fibrosis (bile duct ligation and thioacetamide (TAA)-induced)<sup>42</sup>.

## Kidney fibrosis

$\alpha v \beta 6$  integrin expression is low in the normal kidney, but marked induction of this integrin occurs in a wide range of renal diseases associated with chronic inflammation and fibrosis. Human biopsy samples from membranous glomerulonephritis, diabetes mellitus, IgA nephropathy, Goodpasture's syndrome, Alport syndrome and lupus all demonstrated prominent  $\alpha v \beta 6$  staining in the epithelial lining of dilated and damaged tubules<sup>43</sup>. To assess the potential regulatory role of  $\alpha v \beta 6$  in renal fibrosis, Hahm et al. investigated the effects of function-blocking  $\alpha v \beta 6$  antibodies and genetic ablation of the  $\beta 6$  subunit using a mouse model of Alport syndrome ( $Col4A3^{-/-}$  mice).  $\alpha v \beta 6$ -blocking antibody treatment attenuated accumulation of activated fibroblasts and deposition of interstitial collagen matrix, and similar inhibition of renal fibrosis was observed in  $\beta 6$ -deficient Alport mice. Renal fibrosis is also decreased in  $\beta 6$  null mice following unilateral ureteric obstruction<sup>44</sup>, further demonstrating that  $\alpha v \beta 6$  plays a central regulatory role in the pathogenesis of kidney fibrosis.

## Skin fibrosis

In recent years there have been a number of studies focusing on the role of the  $\alpha v$  integrins in skin fibrosis and wound healing. Systemic sclerosis or scleroderma is an acquired disease typically leading to fibrosis of the skin and internal organs<sup>3</sup>.  $\alpha v \beta 3$  and  $\alpha v \beta 5$  expression are upregulated on human scleroderma fibroblasts and both of these integrins are involved in



activation of latent TGF $\beta$ 1 in primary cultures of these cells. Furthermore, treatment of scleroderma fibroblasts with anti- $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5 antibodies reduced type I procollagen expression<sup>8,9,45,46,47</sup>. A role for  $\alpha$ v $\beta$ 6 in skin wound healing has also been examined.  $\alpha$ v $\beta$ 6 expression is strongly upregulated in the epidermis of human chronic wounds. Furthermore, transgenic mice harboring the human  $\beta$ 6 integrin gene under the control of the cytokeratin 14 promoter (to target constitutive expression of the  $\alpha$ v $\beta$ 6 integrin in epidermal basal cells) develop spontaneous chronic skin wounds surrounded by progressive fibrosis<sup>48</sup>. In addition, aged  $\beta$ 6 null mice demonstrate a significant delay in wound healing when compared to age-matched controls<sup>49</sup>.

Skin scleroderma can be modeled in mice by repetitive subcutaneous injection of bleomycin. To investigate the role of  $\beta$ 1 integrin in cutaneous sclerosis Liu et al. generated mice with fibroblast specific deletion of the  $\beta$ 1 integrin (using mice expressing a tamoxifen-inducible Cre recombinase driven by the mouse collagen type 1, alpha 2 promoter)<sup>50</sup>. Bleomycin treatment induced marked cutaneous thickening and fibrosis in control mice, however fibroblast specific deletion of  $\beta$ 1 integrins resulted in resistance to bleomycin-induced skin fibrosis.

Table 1 summarizes the murine fibrosis models which have demonstrated a role for integrins in the regulation of fibrosis *in vivo*. Clearly these studies cannot be translated directly to human disease, but they do offer very useful insights into the molecular mechanisms driving fibrosis, allowing potential therapeutic targets to be identified.

## Therapeutic targeting of integrins

Although TGF $\beta$ 1 is a promising target for the treatment of fibrotic diseases, all of the currently available methods for inhibiting TGF $\beta$  target all three mammalian isoforms. TGF $\beta$  inhibitors therefore have the potential for important unintended side effects. One concern relates to the potential for carcinogenesis as TGF $\beta$ 1 has an anti-proliferative effect on most epithelial cell types. This is particularly relevant with regard to advanced liver fibrosis in humans, as most hepatocellular carcinomas originate from underlying cirrhotic liver tissue. Secondly, owing to the critical role of TGF $\beta$ 1 in immunosuppression (TGF $\beta$ 1 null mice die at an early age from massive multi-organ inflammation<sup>51,52</sup>, generalized blockade of TGF $\beta$  activity may also lead to excessive autoimmunity and inflammation which could be highly detrimental in a patient with advanced fibrosis and limited organ reserve. Therefore inhibition of TGF $\beta$ 1 signaling at specific sites, via inhibition of specific integrins, may yield the desired anti-fibrotic effects without the unwanted side-effects of pan-TGF $\beta$  blockade.

Specific blocking antibodies to  $\alpha$ v $\beta$ 6 have shown therapeutic promise in a wide range of pre-clinical models of fibrosis including lung fibrosis<sup>20,53</sup>, renal fibrosis<sup>43,44</sup> and peri-biliary fibrosis<sup>37,54</sup>. Furthermore, in the lung low doses of  $\alpha$ v $\beta$ 6 blocking antibodies can prevent bleomycin-induced or radiation-induced pulmonary fibrosis in mice, without causing inflammation<sup>20,53</sup>. A monoclonal antibody targeting  $\alpha$ v $\beta$ 6 (clone 6.3G9) has been humanized as STX-100, and is currently being evaluated in phase 2 clinical trials for the treatment of patients with idiopathic pulmonary fibrosis. As noted above, pre-clinical data also suggest that targeting  $\alpha$ 3 $\beta$ 1,  $\alpha$ v $\beta$ 3,  $\alpha$ v $\beta$ 5,  $\alpha$ v $\beta$ 8 or the  $\beta$ 1 integrin on fibroblasts that regulate cutaneous fibrosis could hold promise for treatment of fibrotic diseases, however much less is currently known about the risk/benefit ratios of any of these interventions.

## Conclusions

In recent years it has become apparent that integrins have profound effects on fibrosis in multiple organs. There is now abundant *in vivo* data demonstrating critical regulatory roles for integrins expressed on different cell types during the fibrotic process. The component

parts of tissue fibrogenesis are exquisitely complex, and these studies highlight the important cross-talk between epithelia, tissue myofibroblasts and the cells of the immune system during the evolution and resolution of fibrosis. Strategies to manipulate integrins, such as antibody blockade and small molecule inhibitors, will hopefully yield effective anti-fibrotic therapies.

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## References

1. Wynn TA. Integrating mechanisms of pulmonary fibrosis. *J Exp Med*. 2011; 208:1339–1350. [PubMed: 21727191]
2. Henderson NC, Iredale JP. Liver fibrosis: cellular mechanisms of progression and resolution. *Clin Sci (Lond)*. 2007; 112:265–280. [PubMed: 17261089]
3. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest*. 2007; 117:557–567. [PubMed: 17332883]
4. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell*. 2002; 110:673–687. [PubMed: 12297042]
5. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell*. 1992; 69:11–25. [PubMed: 1555235]
6. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, Rifkin DB, Sheppard D. The integrin  $\alpha v \beta 6$  binds and activates latent TGF $\beta 1$ : a mechanism for regulating pulmonary inflammation and fibrosis. *Cell*. 1999; 96:319–328. [PubMed: 10025398]
7. Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, Sheppard D, Broaddus VC, Nishimura SL. The integrin  $\alpha v \beta 8$  mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF- $\beta 1$ . *J Cell Biol*. 2002; 157:493–507. [PubMed: 11970960]
8. Asano Y, Ihn H, Yamane K, Jinnin M, Mimura Y, Tamaki K. Increased expression of integrin  $\alpha v \beta 3$  contributes to the establishment of autocrine TGF- $\beta$  signaling in scleroderma fibroblasts. *J Immunol*. 2005; 175:7708–7718. [PubMed: 16301681]
9. Asano Y, Ihn H, Yamane K, Jinnin M, Tamaki K. Increased expression of integrin  $\alpha v \beta 5$  induces the myofibroblastic differentiation of dermal fibroblasts. *Am J Pathol*. 2006; 168:499–510. [PubMed: 16436664]
10. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF- $\beta 1$  from the extracellular matrix. *J Cell Biol*. 2007; 179:1311–1323. [PubMed: 18086923]
11. Ignatz RA, Massagué J. Transforming growth factor- $\beta$  stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem*. 1986; 261:4337–4345. [PubMed: 3456347]
12. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci AS. Transforming growth factor type  $\beta$ : rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci U S A*. 1986; 83:4167–4171. [PubMed: 2424019]
13. Leask A, Abraham DJ. TGF- $\beta$  signaling and the fibrotic response. *FASEB J*. 2004; 18:816–827. [PubMed: 15117886]
14. Gleizes PE, Munger JS, Nunes I, Harpel JG, Mazzieri R, Noguera I, Rifkin DB. TGF- $\beta$  latency: biological significance and mechanisms of activation. *Stem Cells*. 1997; 15:190–197. [PubMed: 9170210]
15. Munger JS, Harpel JG, Gleizes PE, Mazzieri R, Nunes I, Rifkin DB. Latent transforming growth factor- $\beta$ : structural features and mechanisms of activation. *Kidney Int*. 1997; 51:1376–1382. [PubMed: 9150447]



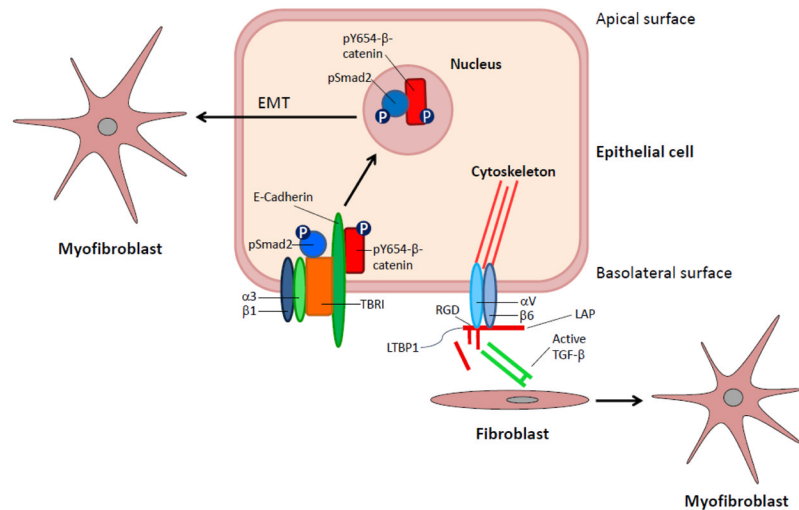
16. Annes JP, Rifkin DB, Munger JS. The integrin  $\alpha$ V $\beta$ 6 binds and activates latent TGF $\beta$ 3. *FEBS Lett.* 2002; 511:65–68. [PubMed: 11821050]
17. Aluwihare P, Mu Z, Zhao Z, Yu D, Weinreb PH, Horan GS, Violette SM, Munger JS. Mice that lack activity of  $\alpha$ v $\beta$ 6- and  $\alpha$ v $\beta$ 8-integrins reproduce the abnormalities of Tgfb1- and Tgfb3-null mice. *J Cell Sci.* 2009; 122:227–232. [PubMed: 19118215]
18. Breuss JM, Gillett N, Lu L, Sheppard D, Pytela R. Restricted distribution of integrin beta 6 mRNA in primate epithelial tissues. *J Histochem Cytochem.* 1993; 41:1521–1527. [PubMed: 8245410]
19. Huang XZ, Wu JF, Cass D, Erle DJ, Corry D, Young SG, Farese RV, Sheppard D. Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. *J Cell Biol.* 1996; 133:921–928. [PubMed: 8666675]
20. Puthawala K, Hadjiangelis N, Jacoby SC, Bayongan E, Zhao Z, Yang Z, Devitt ML, Horan GS, Weinreb PH, Lukashov ME, Violette SM, Grant KS, Colarossi C, Formenti SC, Munger JS. Inhibition of integrin  $\alpha$ (v) $\beta$ 6, an activator of latent transforming growth factor- $\beta$ , prevents radiation-induced lung fibrosis. *Am J Respir Crit Care Med.* 2008; 177:82–90. [PubMed: 17916808]
21. Kaminski N, Allard JD, Pittet JF, Zuo F, Griffiths MJ, Morris D, Huang X, Sheppard D, Heller RA. Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. *Proc Natl Acad Sci U S A.* 2000; 97:1778–1783. [PubMed: 10677534]
22. Jenkins RG, Su X, Su G, Scotton CJ, Camerer E, Laurent GJ, Davis GE, Chambers RC, Matthay MA, Sheppard D. Ligation of protease-activated receptor 1 enhances  $\alpha$ (v) $\beta$ 6 integrin-dependent TGF- $\beta$  activation and promotes acute lung injury. *J Clin Invest.* 2006; 116:1606–1614. [PubMed: 16710477]
23. Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, Springer TA. Latent TGF- $\beta$  structure and activation. *Nature.* 2011; 474:343–349. [PubMed: 21677751]
24. Zhu J, Motejlek K, Wang D, Zang K, Schmidt A, Reichardt LF.  $\beta$ 8 integrins are required for vascular morphogenesis in mouse embryos. *Development.* 2002; 129:2891–2903. [PubMed: 12050137]
25. Proetzel G, Pawlowski SA, Wiles MV, Yin M, Boivin GP, Howles PN, Ding J, Ferguson MW, Doetschman T. Transforming growth factor- $\beta$  3 is required for secondary palate fusion. *Nat Genet.* 1995; 11:409–414. [PubMed: 7493021]
26. Araya J, Cambier S, Markovics JA, Wolters P, Jablons D, Hill A, Finkbeiner W, Jones K, Broaddus VC, Sheppard D, Barczak A, Xiao Y, Erle DJ, Nishimura SL. Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD patients. *J Clin Invest.* 2007; 117:3551–3562. [PubMed: 17965775]
27. Kitamura H, Cambier S, Somanath S, Barker T, Minagawa S, Markovics J, Goodsell A, Publicover J, Reichardt L, Jablons D, Wolters P, Hill A, Marks JD, Lou J, Pittet JF, Gauldie J, Baron JL, Nishimura SL. Mouse, human lung fibroblasts regulate dendritic cell trafficking, airway inflammation, and fibrosis through integrin  $\alpha$ v $\beta$ 8-mediated activation of TGF- $\beta$ . *J Clin Invest.* 2011; 121:2863–2875. [PubMed: 21646718]
28. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, Belperio JA, Keane MP, Strieter RM. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest.* 2004; 114:438–446. [PubMed: 15286810]
29. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci U S A.* 2006; 103:13180–13185. [PubMed: 16924102]
30. Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM, Borok Z. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor- $\beta$ 1: potential role in idiopathic pulmonary fibrosis. *Am J Pathol.* 2005; 166:1321–1332. [PubMed: 15855634]
31. Chattopadhyay N, Wang Z, Ashman LK, Brady-Kalnay SM, Kreidberg JA.  $\alpha$ 3 $\beta$ 1 integrin-CD151, a component of the cadherin-catenin complex, regulates PTPmu expression and cell-cell adhesion. *J Cell Biol.* 2003; 163:1351–1362. [PubMed: 14691142]

32. Kim KK, Wei Y, Szekeres C, Kugler MC, Wolters PJ, Hill ML, Frank JA, Brumwell AN, Wheeler SE, Kreidberg JA, Chapman HA. Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. *J Clin Invest*. 2009; 119:213–224. [PubMed: 19104148]
33. Rock JR, Barkauskas CE, Cronic MJ, Xue Y, Harris JR, Liang J, Noble PW, Hogan BL. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci U S A*. 2011; 108:1475–1483.
34. Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, Duffield JS. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol*. 2010; 176:85–97. [PubMed: 20008127]
35. Chu AS, Diaz R, Hui JJ, Yanger K, Zong Y, Alpini G, Stanger BZ, Wells RG. Lineage tracing demonstrates no evidence of cholangiocyte epithelial-to-mesenchymal transition in murine models of hepatic fibrosis. *Hepatology*. 2011; 53:1685–1695. [PubMed: 21520179]
36. Popov Y, Patsenker E, Stickel F, Zaks J, Bhaskar KR, Niedobitek G, Kolb A, Friess H, Schuppan D. Integrin alphavbeta6 is a marker of the progression of biliary and portal liver fibrosis and a novel target for antifibrotic therapies. *J Hepatol*. 2008; 48:453–464. [PubMed: 18221819]
37. Wang B, Dolinski BM, Kikuchi N, Leone DR, Peters MG, Weinreb PH, Violette SM, Bissell DM. Role of alphavbeta6 integrin in acute biliary fibrosis. *Hepatology*. 2007; 46:1404–1412. [PubMed: 17924447]
38. Patsenker E, Popov Y, Stickel F, Jonczyk A, Goodman SL, Schuppan D. Inhibition of integrin alphavbeta6 on cholangiocytes blocks transforming growth factor-beta activation and retards biliary fibrosis progression. *Gastroenterology*. 2008; 135:660–670. [PubMed: 18538673]
39. de Leeuw AM, McCarthy SP, Geerts A, Knook DL. Purified rat liver fat-storing cells in culture divide and contain collagen. *Hepatology*. 1984; 4:392–403. [PubMed: 6373550]
40. Friedman SL, Roll FJ, Boyles J, Bissell DM. Hepatic lipocytes: the principal collagen-producing cells of normal rat liver. *Proc Natl Acad Sci U S A*. 1985; 82:8681–8685. [PubMed: 3909149]
41. Zhou X, Murphy FR, Gehdu N, Zhang J, Iredale JP, Benyon RC. Engagement of alphavbeta3 integrin regulates proliferation and apoptosis of hepatic stellate cells. *J Biol Chem*. 2004; 279:23996–24006. [PubMed: 15044441]
42. Patsenker E, Popov Y, Stickel F, Schneider V, Ledermann M, Sagesser H, Niedobitek G, Goodman SL, Schuppan D. Pharmacological inhibition of integrin alphavbeta3 aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. *Hepatology*. 2009; 50:1501–1511. [PubMed: 19725105]
43. Hahm K, Lukashev ME, Luo Y, Yang WJ, Dolinski BM, Weinreb PH, Simon KJ, Chun Wang L, Leone DR, Lobb RR, McCrann DJ, Allaire NE, Horan GS, Fogo A, Kalluri R, Shield CF, Sheppard D, Gardner HA, Violette SM. Alphav beta6 integrin regulates renal fibrosis and inflammation in Alport mouse. *Am J Pathol*. 2007; 170:110–125. [PubMed: 17200187]
44. Ma LJ, Yang H, Gaspert A, Carlesso G, Barty MM, Davidson JM, Sheppard D, Fogo AB. Transforming growth factor-beta-dependent and -independent pathways of induction of tubulointerstitial fibrosis in beta6(–/–) mice. *Am J Pathol*. 2003; 163:1261–1273. [PubMed: 14507636]
45. Asano Y, Ihn H, Yamane K, Kubo M, Tamaki K. Increased expression levels of integrin alphavbeta5 on scleroderma fibroblasts. *Am J Pathol*. 2004; 164:1275–1292. [PubMed: 15039216]
46. Asano Y, Ihn H, Yamane K, Jinnin M, Mimura Y, Tamaki K. Involvement of alpha5beta5 integrin-mediated activation of latent transforming growth factor beta1 in autocrine transforming growth factor beta signalling in systemic sclerosis fibroblasts. *Arthritis & Rheumatism*. 2005; 52:2897–2905. [PubMed: 16142753]
47. Asano Y, Ihn H, Jinnin M, Mimura Y, Tamaki K. Involvement of alphavbeta5 integrin in the establishment of autocrine TGF-beta signaling in dermal fibroblasts derived from localized scleroderma. *J Invest Dermatol*. 2006; 126:1761–1769. [PubMed: 16675963]
48. Häkkinen L, Koivisto L, Gardner H, Saarialho-Kere U, Carroll JM, Lakso M, Rauvala H, Laato M, Heino J, Larjava H. Increased expression of beta6-integrin in skin leads to spontaneous development of chronic wounds. *Am J Pathol*. 2004; 164:229–242. [PubMed: 14695336]

49. AlDahlawi S, Eslami A, Häkkinen L, Larjava HS. The alphavbeta6 integrin plays a role in compromised epidermal wound healing. *Wound Repair Regen.* 2006; 14:289–297. [PubMed: 16808807]
50. Liu S, Kapoor M, Denton CP, Abraham DJ, Leask A. Loss of beta1 integrin in mouse fibroblasts results in resistance to skin scleroderma in a mouse model. *Arthritis Rheum.* 2009; 60:2817–2821. [PubMed: 19714619]
51. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Annunziata N, Doetschman T. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature.* 1992; 359:693–699. [PubMed: 1436033]
52. Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci U S A.* 1993; 90:770–774. [PubMed: 8421714]
53. Horan GS, Wood S, Ona V, Li DJ, Lukashev ME, Weinreb PH, Simon KJ, Hahm K, Allaire NE, Rinaldi NJ, Goyal J, Feghali-Bostwick CA, Matteson EL, O'Hara C, Lafyatis R, Davis GS, Huang X, Sheppard D, Violette SM. Partial inhibition of integrin alpha(v)beta6 prevents pulmonary fibrosis without exacerbating inflammation. *Am J Respir Crit Care Med.* 2008; 177:56–65. [PubMed: 17916809]
54. Sullivan BP, Weinreb PH, Violette SM, Luyendyk JP. The coagulation system contributes to alphaVbeta6 integrin expression and liver fibrosis induced by cholestasis. *Am J Pathol.* 2010; 177:2837–2849. [PubMed: 21037076]

### Highlights

1. Tissue fibrosis is a major healthcare burden worldwide.
2. Integrin-mediated activation of latent TGF $\beta$  is a major mechanism driving fibrosis.
3. Pharmacologic manipulation of integrins may lead to new antifibrotic treatments.



**Figure 1. Mechanisms of integrin-mediated regulation of lung fibrosis**

$\alpha 3\beta 1$ -mediated promotion of myofibroblast formation: In uninjured alveolar epithelial cells  $\alpha 3\beta 1$  co-localises with TGF $\beta$  receptor I (TBRI), E-cadherin and  $\beta$ -catenin. In the presence of TGF $\beta 1$ ,  $\alpha 3$  integrin is required for  $\beta$ -catenin phosphorylation at tyrosine residue 654 (Y654), which is necessary for formation of a pY654- $\beta$ -catenin/p-SMAD2 complex. This pY654- $\beta$ -catenin/p-SMAD2 complex then translocates to the nucleus and induces EMT (epithelial-mesenchymal transition).  $\alpha v\beta 6$ -mediated activation of latent TGF $\beta$ :  $\alpha v\beta 6$  binds to the RGD sequence in the LAP of TGF $\beta 1$  and 3. This complex is tethered by a disulfide linkage to LTBP1, which is essential for TGF $\beta$  activation. Binding alone is insufficient to activate latent complexes. Activation requires extracellular signals that lead to epithelial cell contraction and induction of a conformational change in the latent complex. This conformational change presents the active site on the mature TGF $\beta$  dimer to TGF $\beta$  receptors on adjacent cells, such as fibroblasts.

Table 1

Analysis of integrin function in transgenic mouse models of fibrosis

Integrin	Organ	Model of fibrosis	Manipulation	Summary	Reference
$\alpha.v\beta6$	Lung	Bleomycin	Global knockout of $\alpha.v\beta6$	Absence of $\beta6$ markedly attenuates bleomycin-induced lung fibrosis via a reduction in activation of latent TGF $\beta1$	Munger et al. (1999) <sup>6</sup>
$\alpha.v\beta6$	Lung	Radiation	Global knockout of $\alpha.v\beta6$	Absence of $\beta6$ protects against radiation-induced lung fibrosis	Puthawala et al.(2008) (2008) <sup>20</sup>
$\alpha.v\beta6$	Kidney	Mouse model of Alport syndrome (Col4A3 <sup>-/-</sup> mice)	Global knockout of $\alpha.v\beta6$	Absence of $\beta6$ attenuates renal fibrosis in $\beta6$ -deficient Alport mice	Hahm et al. (2007) <sup>43</sup>
$\alpha.v\beta6$	Kidney	Unilateral ureteric obstruction (UUO)	Global knockout of $\alpha.v\beta6$	Absence of $\beta6$ attenuates UUO-induced renal fibrosis	Ma et al. (2003) <sup>44</sup>
$\alpha.v\beta6$	Liver	Bile duct ligation	Global knockout of $\alpha.v\beta6$	Absence of $\beta6$ attenuates acute biliary fibrosis	Wang et al. (2007) <sup>37</sup>
$\alpha.v\beta8$	Lung	IL-1 $\beta$ and allergen induced lung injury	Conditional knockout of $\alpha.v\beta8$ in fibroblasts	Reduced airway remodelling and dampening of adaptive immunity	Kitamura et al. (2011) <sup>27</sup>
$\alpha.3\beta1$	Lung	Bleomycin	Conditional knockout of $\alpha.3\beta1$ in epithelial cells	Decreased lung fibrosis via a reduction in $\beta$ -catenin/Smad signalling and EMT	Kim et al. (2009) <sup>32</sup>
$\beta1$	Skin	Subcutaneous injection of bleomycin	Conditional knockout of $\beta1$ in fibroblasts	Deletion of $\beta1$ integrin protects against bleomycin-induced skin fibrosis	Liu et al. (2009) <sup>50</sup>