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# $\alpha$ v integrins: key regulators of tissue fibrosis

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**Abstract** Chronic tissue injury with fibrosis results in the disruption of tissue architecture, organ dysfunction and eventual organ failure. Therefore, the development of effective anti-fibrotic therapies is urgently required. During fibrogenesis, complex interplay occurs between cellular and extracellular matrix components of the wound healing response. Integrins, a family of transmembrane cell adhesion molecules, play a key role in mediating intercellular and cell-matrix interactions. Thus, integrins provide a major node of communication between the extracellular matrix, inflammatory cells, fibroblasts and parenchymal cells and, as such, are intimately involved in the initiation, maintenance and resolution of tissue fibrosis. Modulation of members of the  $\alpha$ v integrin family has exhibited profound effects on fibrosis in multiple organs and disease states. In this review, we discuss the current knowledge of the mechanisms of  $\alpha$ v-integrin-mediated regulation of fibrogenesis and show that the therapeutic targeting of specific  $\alpha$ v integrins represents a promising avenue to treat patients with a broad range of fibrotic diseases.

**Keywords** Integrins · Fibrosis · TGF $\beta$  · Extracellular matrix · Myofibroblasts

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## Introduction

Chronic tissue injury with fibrosis results in the disruption of tissue architecture, organ dysfunction and eventual organ failure. Although fibrosis represents a massive healthcare burden worldwide, currently available therapeutic options are severely limited and organ transplantation is, in many cases, the only effective treatment for end-stage fibrotic disease. However, limited donor organ availability, high cost and comorbidities in potential recipients mean that, on a global scale, organ transplantation can only be offered to a small percentage of patients suffering from the complications of fibrosis. The development of effective anti-fibrotic therapies is therefore essential to improving our patient care.

Fibrogenesis involves a complex interplay between the inflammatory, epithelial, myofibroblast and extracellular matrix (ECM) components of the wound healing response (Henderson and Iredale 2007; Varga and Abraham 2007). The pericellular ECM, in particular, represents a highly dynamic environment known to exert profound influences on cell behaviour. As transmembrane proteins, the integrin family of cell adhesion molecules mediates many key cell-cell and cell-matrix interactions during fibrosis. Integrins represent a major node of communication between the ECM, inflammatory cells, fibroblasts and parenchymal cells and are intimately involved in the initiation, maintenance and resolution of tissue fibrosis. Integrins are composed of non-covalent  $\alpha/\beta$  heterodimers of which there are 24 known members in humans and comprise 18 different  $\alpha$  subunits and 8  $\beta$  subunits. They can translate extracellular signals, resulting in a wide range of downstream effects on cell adhesion, migration, proliferation, differentiation and apoptosis (Hynes 2002). Of particular note is the  $\alpha$ v subunit, which forms heterodimers with the  $\beta$ 1,  $\beta$ 3,  $\beta$ 5,  $\beta$ 6 or  $\beta$ 8 subunits. As outlined in Table 1, modulation of various members of the  $\alpha$ v integrin family has exhibited

**Table 1** Effects of  $\alpha$ v integrin inhibition in pre-clinical models of fibrosis (*CCL<sub>4</sub>* carbon tetrachloride, *BDL* bile duct ligation, *TAA* thioacetamide, *UUO* unilateral ureteric obstruction, *TGF* transforming growth factor, *DDC* 3,5-diethoxycarbonyl-1,4-dihydrocollidine, *IL-1 $\beta$*  interleukin-1 $\beta$ )

$\alpha$ v integrin subunit	Organ	Model	Method of inhibition	Summary	Reference
$\beta$ 1	Liver lung	<i>CCL<sub>4</sub></i>	Small molecule inhibitor, c8	Inhibition of $\alpha$ v $\beta$ 1 significantly reduced established fibrosis in liver and lung	Reed et al. 2015
$\beta$ 3/ $\beta$ 5	Liver	Bleomycin BDL/TAA	Small molecule inhibitor, Cilengitide	Increased hepatic collagen deposition and pro-fibrogenic gene expression	Patsenker et al. 2009
$\beta$ 3/ $\beta$ 5	Lung	Bleomycin	Genetic double knockout	No protection from lung fibrosis	Atabai et al. 2009
$\beta$ 3	Skin	Fibrillin-1 mutation	Genetic knockout mice	Knockout of $\beta$ 3 rescued progression of skin stiffness and reduced collagen deposition	Gerber et al. 2015
$\beta$ 6	Kidney	Mouse model of Alport syndrome ( <i>Col4A3<sup>-/-</sup></i> mice)	Function-blocking $\alpha$ v $\beta$ 6 monoclonal antibodies	$\beta$ 6 knockout reduced renal fibrosis development in $\beta$ 6-deficient Alport mice	Hahm et al. 2007
$\beta$ 6	Kidney	UUO	$\beta$ 6 knockout mice $\beta$ 6 knockout mice	UUO-induced renal fibrosis is attenuated in $\beta$ 6-knockout mice	Ma et al. 2003
$\beta$ 6	Lung	TGF $\alpha$ over-expression	$\beta$ 6 function-blocking monoclonal antibody, 6.3G9 $\beta$ 6 knockout mice	Inhibition and genetic depletion in established fibrosis attenuated the continuation of pleural thickening and decline in lung function	Madala et al. 2014
$\beta$ 6	Lung	Bleomycin	$\beta$ 6 knockout mice.	The LAP of TGF $\beta$ is a ligand for integrin $\alpha$ v $\beta$ 6, and it can also bind and activate TGF $\beta$ , locally regulating its function.	(Munger et al. 1999)
$\beta$ 6	Liver	BDL	Antibody, 3G9	Reduced acute biliary fibrosis	Wang et al. 2007
$\beta$ 6	Liver	BDL	Small molecule inhibitor, EMD527040	Inhibition reduced bile duct proliferation and peribiliary collagen deposition and increased fibrolytic gene expression	Patsenker et al. 2008
$\beta$ 6	Liver	DDC	$\beta$ 6 function-blocking monoclonal antibody, 3G9 and $\beta$ 6 knockout mice	Biliary fibrosis rescued by anti- $\alpha$ v $\beta$ 6 antibody treatment through inhibition of progenitor cell expansion	Peng et al. 2015
$\beta$ 6	Lung	Radiation	$\beta$ 6 function-blocking monoclonal antibody, 6.3G9 and $\beta$ 6 knockout mice	Low dose prevented fibrogenesis; however, higher doses resulted in lung inflammation	Puthawala et al. 2008
$\beta$ 6	Lung	Bleomycin	$\beta$ 6 function-blocking monoclonal antibody, 6.3G9 and $\beta$ 6 knockout mice	Attenuation of lung fibrosis and reduced TGF $\beta$ activity	Horan et al. 2008
$\beta$ 8	Lung	IL-1 $\beta$ and allergen-induced lung injury	Conditional knockout of $\alpha$ v $\beta$ 8 on fibroblasts	Inhibition of airway fibrosis in both models of lung injury	Kitamura et al. 2011

*CCL<sub>4</sub>* (carbon tetrachloride), *BDL* (bile duct ligation), *TAA* (thioacetamide), *UUO* (unilateral ureteric obstruction), *TGF* (transforming growth factor), *DDC* (3,5-diethoxycarbonyl-1,4-dihydrocollidine), *IL-1 $\beta$*  (interleukin-1 $\beta$ ), *LAP* (latency associated peptide)

profound effects on fibrosis in multiple organs and disease states. In this review, we will discuss the ways in which  $\alpha$ v integrins regulate the fibrotic process and show that the therapeutic targeting of specific  $\alpha$ v integrins represents a promising avenue for treating patients with a broad range of fibrotic diseases.

### Regulation of TGF $\beta$ activity by $\alpha$ v integrins

In addition to their direct effects on cellular proliferation and survival, integrins may also potentiate signals from soluble growth and survival factors. Secreted transforming growth factor beta (TGF $\beta$ ) is a key regulator of fibrosis in multiple organs (Ignatz and Massagué 1986; Roberts et al. 1986; Hynes 2002; Leask and Abraham 2004). The three mammalian isoforms of TGF $\beta$  are all synthesised as precursor proteins, which are then processed by proteolytic cleavage within the endoplasmic reticulum. They are subsequently assembled as a non-covalent “small latent complex” of a disulfide-linked homodimer of the mature cytokine (a short C-terminal fragment), which is encased within 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 by holding it in a conformation distinct from that of the free dimer. This “small latent complex” is further modified in the endoplasmic reticulum by disulfide linkage to another family of proteins, namely latent TGF $\beta$  binding proteins, which upon secretion are themselves chemically cross-linked to the ECM, for storing and tethering TGF $\beta$  in a latent form in the extracellular space. Tissue forces such as cellular contraction disrupt the latency cage, releasing the mature dimer and enabling it to activate TGF $\beta$  receptors. Much of the regulation of TGF $\beta$  biology thus occurs at the level of extracellular activation of this stored latent complex (Gleizes et al. 1997; Munger et al. 1997).

The three isoforms of TGF $\beta$ , namely TGF $\beta$ -1, -2 and -3, appear to have overlapping functions. All mediate their effects, at least in part, through the intracellular SMAD pathway and, of the three, TGF $\beta$ 1 is the most widely involved in fibrogenesis.  $\alpha$ v integrins have been demonstrated to play a key role in the activation of latent TGF $\beta$ 1 and TGF $\beta$ 3 (Annes et al. 2002). Specifically, all five integrins have been shown to interact with a linear arginine-glycine-aspartic acid (RGD) motif present in the LAP, activating latent TGF- $\beta$  (Munger et al. 1999; Mu et al. 2002; Asano et al. 2006; Wipff et al. 2007). Inhibition and blockade of  $\alpha$ v $\beta$ 6 and  $\alpha$ v $\beta$ 8 phenocopies all have the developmental effects of loss of TGF $\beta$ 1 and 3 (Aluwihare et al. 2008), 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 underlying its contribution to adult tissue pathology are less well understood.

The actin cytoskeleton also plays a role in the activation of TGF $\beta$ 1, as demonstrated by the inhibition of TGF $\beta$  activity following the blockade of actin polymerisation (Munger et al. 1997) or the inhibition of Rho kinase (Jenkins et al. 2006). The mechanical force generated by integrin-mediated regulation of the actin cytoskeleton is a common mechanism for activating latent TGF $\beta$ 1 (Shi et al. 2011). Shi et al. (2011) reported that mere complex formation between integrin  $\alpha$ v $\beta$ 6 and the prodomain of TGF $\beta$ 1 is insufficient for its release, with the activation of TGF $\beta$ 1 requiring a further force-dependent unfastening of a “straitjacket” that encircles each growth factor dimer. This is attributable to the LAP prodomain altering the conformation of TGF $\beta$ 1 and effectively shielding TGF $\beta$ 1 from recognition by receptors.

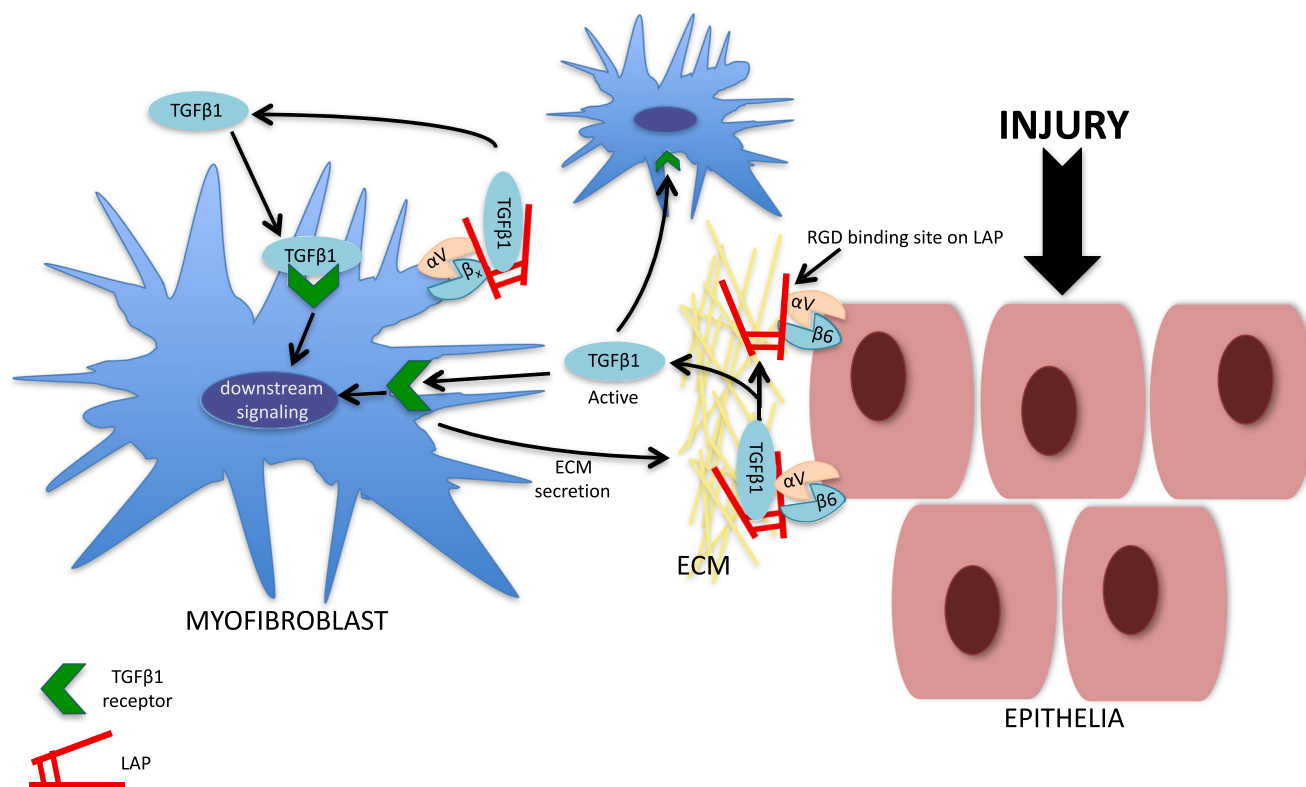
One cell type intrinsically involved in organ scarring is the myofibroblast that provides a major source of ECM proteins during fibrogenesis (Klingberg et al. 2012). The precise origin of myofibroblasts is unclear with studies indicating transdifferentiation from both local and influxing cells in response to growth factors and mechanical tension (Munger et al. 1999; Aluwihare et al. 2008; Iwaisako et al. 2014). Highly contractile cells, myofibroblasts express several  $\alpha$ v integrins that transmit the force generated by the actin cytoskeleton to the ECM (Fig. 1). Myofibroblast  $\alpha$ v integrins are able to liberate and thereby activate TGF $\beta$ 1 deposits in the ECM via mechanical force. Further insights into this process have been provided by Klingberg et al. (2014) who demonstrated, through a series of in vitro experiments, that latent ECM-bound TGF $\beta$ 1 is primed by the stiffening of the surrounding ECM, such that greater amounts are released compared with a relaxed ECM (Klingberg et al. 2014). Therefore, prior to force-mediated activation of TGF $\beta$ 1, myofibroblasts actively re-organise the ECM, increasing the bioavailability of the bound latent TGF $\beta$ 1 complex.

### Role of individual $\alpha$ v integrins during fibrogenesis

#### $\alpha$ v $\beta$ 1

Study of the function of  $\alpha$ v $\beta$ 1 during fibrogenesis has been challenging. The  $\beta$ 1 integrin dimerises with multiple  $\alpha$  subunits and, therefore, interrogation of the role of  $\alpha$ v $\beta$ 1 has not been possible by using standard transgenic mouse approaches. This has further been hampered by a lack of specific function-blocking antibodies or small molecule antagonists. Recently, however, a small molecule inhibitor of  $\alpha$ v $\beta$ 1, which is highly specific and potent in its activity, has been developed (Reed et al. 2015).

To develop this  $\alpha$ v $\beta$ 1 small molecule inhibitor, Reed et al. (2015) used the known base compound of  $\alpha$ v and combined



**Fig. 1** Complex interplay of  $\alpha_v$ -integrin-mediated regulation of tissue fibrosis.  $\alpha_v$  integrins ( $\beta_1, \beta_3, \beta_5, \beta_8$ ) expressed on fibroblasts and  $\alpha_v\beta_6$  expressed on epithelia activate transforming growth factor beta (*TGF $\beta$* ) through their interaction with a linear arginine-glycine-aspartic acid (*RGD*) binding motif present on the latency-associated peptide (*LAP*) in

the extracellular matrix (*ECM*). TGF $\beta$  released from the ECM by injured epithelia might directly signal to the myofibroblast to promote further ECM production. Furthermore,  $\alpha_v$  integrins on myofibroblasts can release active TGF $\beta$  from the ECM; this TGF $\beta$  then signals in an autocrine manner to drive further ECM production by myofibroblasts

this with their previously described sulfonamidoproline moiety, which binds to the  $\beta_1$  subunit of  $\alpha_2\beta_1$  (Miller et al. 2009). The efficacy of a set of synthesised compounds in binding to individual  $\alpha_v$  integrin heterodimers and inhibiting cell adhesion was tested by using a panel of cell lines and ligands. Two compounds (c6 and c8) were found to achieve potent and specific inhibition of  $\alpha_v\beta_1$  in vitro, supported by the loss of adhesion to the  $\alpha_v\beta_1$  ligand, fibronectin. In cell adhesion assays in vitro, the c8 compound efficiently blocked the adhesion of fibroblasts to TGF $\beta$ 1 LAP, a result that was not replicated by using antibodies to  $\alpha_v\beta_3, \alpha_v\beta_5, \alpha_v\beta_6$  or  $\alpha_v\beta_8$ . This demonstrated that  $\alpha_v\beta_1$  is the predominant fibroblastic  $\alpha_v$  integrin on fibroblasts mediating adhesion to the TGF $\beta$ 1 LAP. Further in vitro characterisation, by using primary murine hepatic stellate cells and human lung fibroblasts, showed that c8 could potently and specifically inhibit TGF $\beta$  activation.

Using two murine models of fibrosis, namely carbon tetrachloride-induced liver fibrosis and bleomycin-induced lung fibrosis, Reed et al. (2015) demonstrated in vivo that the systemic delivery of c8 reduces fibrosis. Moreover, the degree of protection is comparable to that seen in a previous study, which conditionally deleted all  $\alpha_v$  integrins on myofibroblasts (Henderson et al. 2013) providing further evidence that  $\alpha_v\beta_1$  is, to a large degree, responsible. Direct

evidence of suppressed TGF $\beta$  signalling through reduced phosphorylation of the TGF $\beta$  intracellular signalling effector, Smad3, further suggests that this antifibrotic effect is attributable to the inhibition of  $\alpha_v\beta_1$ -mediated TGF $\beta$  activation.

### $\alpha_v\beta_3$ and $\alpha_v\beta_5$

Expression of  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  is upregulated during cardiac and skin fibrosis and both heterodimers have been reported to activate TGF $\beta$ 1 signalling in vitro (Asano et al. 2004; Jenkins et al. 2006; Shi et al. 2011; Sarrazy et al. 2014). Treatment of primary cultures of scleroderma fibroblasts with anti- $\alpha_v\beta_3$  and anti- $\alpha_v\beta_5$  antibodies reduced type I procollagen expression, suggesting an anti-fibrotic effect (Asano et al. 2005, 2006; Miller et al. 2009). Furthermore, blockade of  $\alpha_v\beta_3$  on culture-activated liver myofibroblasts inhibited their proliferation and increased apoptosis (Zhou et al. 2004).

However, the in vivo effects of  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  inhibition studied via genetic depletion and pharmacological blockade are less consistent. A  $\beta_3/\beta_5$  double-knockout mouse was not protected from bleomycin-induced lung fibrosis, indicating the possible compensatory involvement of other integrins (Atabai et al. 2009). In the thioacetamide (TAA) and bile duct ligation (BDL) liver fibrosis models,  $\alpha_v\beta_3$  inhibition



augmented rather than reduced collagen levels (Patsenker et al. 2009). Furthermore, the  $\alpha\beta3$  antagonist Cilengitide failed to show any anti-fibrotic effects in two distinct models of liver fibrosis (BDL and TAA-induced injury), instead significantly increasing collagen deposition (by 31 % in BDL and 27 % in TAA) and up-regulating pro-fibrotic gene expression (Patsenker et al. 2009). Using a mouse model of stiff skin syndrome, which is caused by a fibrillin-1 mutation and leads to the development of aggressive skin fibrosis, Gerber et al. (2015) reported that  $\beta3$  integrin is expressed on systemic sclerosis dermal fibroblasts.

In the context of cardiac fibrosis,  $\alpha\beta3$  and  $\alpha\beta5$  have recently been shown to compensate for each other's function with regard to TGF $\beta1$  activation and myofibroblast differentiation (Sarrazy et al. 2014). Through a series of in vitro studies by using transfection and short hairpin RNA to overexpress or knockdown  $\beta3$  and  $\beta5$  on human cardiac fibroblasts, the knockdown of one resulted in the elevated expression of the other. Conversely, the overexpression of either integrin reduced the expression of the other. Individual knock-down of  $\beta3$  and  $\beta5$  integrins only moderately reduced the release of active TGF $\beta1$  and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) expression, whereas the simultaneous knock-down of both integrins led to a significant reduction in TGF $\beta1$  release and  $\alpha$ -SMA expression compared with controls.

### $\alpha\beta6$

Expression of  $\alpha\beta6$  integrin in the uninjured lung, liver and kidney is generally low but, in the context of inflammation and fibrosis,  $\alpha\beta6$  levels increase (Munger et al. 1999; Asano et al. 2005; Wang et al. 2007). Expression of  $\alpha\beta6$  is restricted to cells of the epithelial lineage (Breuss et al. 1995) and mediates the activation of TGF $\beta1$  and TGF $\beta3$  by direct binding of the integrin to the LAP (Annes et al. 2002) at epithelial surfaces (Munger et al. 1999; Shi et al. 2011). Cells expressing  $\alpha\beta6$  activate TGF $\beta1$  in vitro and this can be completely inhibited by  $\beta6$ -blocking antibodies. Further, similar to mice homozygous for a null mutation of TGF $\beta1$ , pan- $\beta6$  integrin subunit knockout mice develop exaggerated inflammatory responses in the lungs and skin, albeit less severe than in TGF $\beta1$  null mice (Huang et al. 1996).

In patients with fibrotic liver disease secondary to a variety of aetiologies (primary biliary cirrhosis, alcohol-induced, hepatitis B and C), integrin  $\alpha\beta6$  mRNA expression is increased and has been found to correlate with fibrosis stage in hepatitis C (Popov et al. 2008). Expression of  $\alpha\beta6$  in human chronic dermal wounds is strongly upregulated and, in the primate lung, alveolar epithelial cell  $\alpha\beta6$  expression is rapidly induced following lung injury (Breuss et al. 1993). In mice expressing the cytokeratin 14 promoter, driving constitutive  $\alpha\beta6$  expression in epidermal basal cells, spontaneous chronic skin wounds develop surrounded by fibrotic tissue

(Häkkinen et al. 2004). Conversely, in aged  $\beta6$ -knockout mice, a significant delay in wound healing is seen compared with aged-matched controls (AlDahlawi et al. 2006), suggesting an important role in the timely resolution of injury.

In the lung,  $\beta6$  null mice develop exaggerated inflammation in response to bleomycin treatment but are dramatically protected from subsequent pulmonary fibrosis (Munger et al. 1999). In  $\beta6$  knockout mice treated with bleomycin, microarray analysis of the lungs identified a large group of TGF $\beta$ -inducible genes that were induced at substantially lower levels than in wild-type control mice (Kaminski et al. 2000; Atabai et al. 2009). Administration of anti- $\alpha\beta6$  antibodies was able to attenuate bleomycin-induced pulmonary fibrosis with a concomitant reduction in TGF $\beta$  activity (Horan et al. 2008; Patsenker et al. 2009). Further,  $\beta6$  inhibition, induced both by genetic knockout and blockade with anti- $\alpha\beta6$  antibodies, was protective in radiation-induced pulmonary fibrosis (Puthawala et al. 2008; Patsenker et al. 2008). However, high doses of anti- $\alpha\beta6$  monoclonal antibody resulted in lung inflammation, similar to  $\alpha\beta6$ -null mice (Puthawala et al. 2008; Sarrazy et al. 2014). A humanised monoclonal antibody targeting  $\alpha\beta6$ , STX-100, has been developed and is currently being evaluated in phase 2 clinical trials for the treatment of idiopathic pulmonary fibrosis.

Recently, using both pharmacological and genetic inhibition of the  $\alpha\beta6$  integrin, Madala et al. (2014) demonstrated that, in a TGF $\alpha$  model of pulmonary fibrosis, the activation of the  $\alpha\beta6$ /TGF $\beta$  pathway has a secondary effect on pleural fibrosis. Doxycycline-induced overexpression of TGF $\alpha$  in lung epithelia generates progressive lung fibrosis. With this model of lung fibrosis, the authors demonstrated that the activation of the  $\alpha\beta6$ /TGF $\beta$  pathway occurred following fibrosis development. Inhibition of  $\alpha\beta6$  by using function-blocking antibodies in established lung fibrosis in TGF $\alpha$ -overexpressing mice was able to attenuate the continuation of pleural thickening and decline in lung function. Further, in TGF $\alpha$ -overexpressing mice with 8 weeks of fibrosis induction, genetic ablation of  $\beta6$  limited the histological and physiological pulmonary changes; however, fibrosis was still significant. This suggests that in this specific model of TGF $\alpha$ -induced lung fibrosis, the effective abrogation of fibrosis might require the targeting of multiple pathways in order to limit fibrogenesis and altered lung physiology.

Integrin  $\alpha\beta6$  has also been shown to play an important role in the progression of biliary fibrosis. In the BDL model of acute biliary fibrosis, Wang et al. (2007) demonstrated that bile duct obstruction markedly increased cholangiocyte  $\alpha\beta6$  expression and, moreover, that biliary fibrosis was reduced in  $\beta6$  integrin null mice by 50 % compared with wild-type controls. Administration of an  $\alpha\beta6$ -blocking antibody significantly decreased acute biliary fibrosis following BDL (Wang et al. 2007). Pharmacological inhibition of  $\alpha\beta6$  by using the small molecule inhibitor EMD527040 also inhibited biliary fibrosis. In BDL rats and in Mdr2 (abc4)<sup>-/-</sup> mice (a model of bile duct obstruction), treatment with EMD527040

during established fibrosis reduced bile duct proliferation and peribiliary collagen deposition by between 40 and 50 %, decreased pro-fibrotic gene expression and up-regulated fibrolytic genes (Patsenker et al. 2008). Furthermore,  $\alpha\text{v}\beta\text{6}$  has recently been reported to be expressed on and to regulate the function of hepatic progenitor cells (Peng et al. 2015). Genetic modulation and pharmacological inhibition of  $\alpha\text{v}\beta\text{6}$  activity during chronic liver injury strongly suppressed progenitor cell proliferation in vitro. In vivo, this led to a marked attenuation of biliary fibrogenesis and protection from fibrosis-associated liver cancer (Breuss et al. 1993; Peng et al. 2015).

In renal fibrosis, the regulatory role of  $\alpha\text{v}\beta\text{6}$  has been investigated by using both function-blocking  $\alpha\text{v}\beta\text{6}$  antibodies and the mouse model of Alport syndrome (Col4A3<sup>-/-</sup> mice) combined with  $\beta\text{6}$  integrin knockout mice (Hahm et al. 2007). Treatment with  $\alpha\text{v}\beta\text{6}$ -blocking antibody prevented the accumulation of activated fibroblasts and reduced interstitial collagen matrix deposition. Genetic ablation of  $\beta\text{6}$  integrin, in a model of Alport syndrome (Col4A3<sup>-/-</sup> mice), resulted in similar effects on renal fibrosis. These findings were recapitulated in the unilateral ureteric obstruction model of renal fibrosis (Ma et al. 2003). Although these data suggest a strong regulatory role for  $\alpha\text{v}\beta\text{6}$  in renal fibrosis, the blockade of  $\alpha\text{v}\beta\text{6}$  activity following renal transplantation has been reported to increase significantly the acute rejection of the transplanted organ (Lo et al. 2013).

### $\alpha\text{v}\beta\text{8}$

Mice with  $\beta\text{8}$  subunit knockout exhibit vascular development defects similar to those seen in TGF $\beta\text{1}$  null mice, with a number dying mid-gestation (Zhu et al. 2002). Of those that survive to birth, death occurs soon after from brain haemorrhage, possibly because of the loss of the developmental vascular effects of TGF $\beta\text{1}$ . Furthermore, many of these mice have a cleft palate, a prominent feature in TGF $\beta\text{3}$  knockout mice suggesting an important role for  $\alpha\text{v}\beta\text{8}$  in activating TGF $\beta\text{3}$  in vivo, in addition to TGF $\beta\text{1}$  activation in vivo (Proetzel et al. 1995; Huang et al. 1996).

Both  $\alpha\text{v}\beta\text{8}$  and  $\alpha\text{v}\beta\text{6}$  bind to the same RGD sequence in the LAPs of TGF $\beta\text{1}$  and TGF $\beta\text{3}$ , although the mechanism by which they activate TGF $\beta\text{1}$  has been shown to differ. Whereas  $\alpha\text{v}\beta\text{6}$  requires direct cell–cell contact to activate TGF $\beta\text{1}$ ,  $\alpha\text{v}\beta\text{8}$ -mediated activation requires the extracellular localisation of  $\beta\text{8}$ , in addition to metalloprotease (MMP) activity. Furthermore, in vitro studies have shown that  $\alpha\text{v}\beta\text{8}$  can release active TGF $\beta\text{1}$  into the media (Mu et al. 2002; Popov et al. 2008). Unlike  $\alpha\text{v}\beta\text{6}$ , metalloprotease inhibitors reduce  $\alpha\text{v}\beta\text{8}$ -mediated TGF $\beta\text{1}$  activation and in vitro transfection studies have demonstrated a specific role for MT1-MMP (MMP14) in this process (Breuss et al. 1993; Mu et al. 2002). Araya and colleagues (2007) transfected primary

airway fibroblasts with MMP14 short interfering RNA (siRNA), inhibiting 70 % of its surface expression leading to a marked inhibition of  $\alpha\text{v}\beta\text{8}$ -mediated activation of TGF $\beta$ . This suggests that  $\alpha\text{v}\beta\text{8}$  activates TGF $\beta\text{1}$  by presenting LAP complexes to cell-surface MMPs that, in turn, degrade the LAP, releasing free TGF $\beta\text{1}$ .

In chronic obstructive pulmonary disease (COPD) patients, the degree of airway wall fibrosis has been shown to correlate with  $\alpha\text{v}\beta\text{8}$  expression on isolated airway fibroblasts (Araya et al. 2007). When incubated with interleukin-1 $\beta$  (IL-1 $\beta$ ), lung fibroblasts isolated from COPD patients showed enhanced  $\alpha\text{v}\beta\text{8}$ -dependent TGF $\beta$  activation, collagen expression and pro-inflammatory gene expression compared with normal lung fibroblasts (Araya et al. 2007). Further investigation in vitro showed that the autocrine  $\alpha\text{v}\beta\text{8}$ -mediated activation of TGF $\beta\text{1}$  contributed to the pro-fibrogenic differentiation of COPD fibroblasts, as demonstrated by using siRNA to knockdown  $\beta\text{8}$  expression, leading to reduced  $\alpha\text{SMA}$  and collagen I expression. However, this intervention inhibited only half of the total TGF $\beta$  activation when compared with a TGF $\beta$ -blocking antibody. In murine airway fibrosis, conditional deletion of lung fibroblast  $\alpha\text{v}\beta\text{8}$  inhibited airway fibrosis in both IL-1 $\beta$  and ovalbumin-induced models (Kitamura et al. 2011). Furthermore, depletion of  $\alpha\text{v}\beta\text{8}$  on cultured mouse lung fibroblasts reduced TGF $\beta\text{1}$  activation (Kitamura et al. 2011).

In children with biliary atresia (a pediatric cholestatic disease), immunohistochemical analysis of liver biopsies has shown increased expression of  $\alpha\text{v}\beta\text{8}$  integrin compared with healthy controls (Iordanskaia et al. 2014). Interestingly, dysregulation of MMP14 has also been reported in both human and animal models of biliary atresia (Iordanskaia et al. 2013). This suggests that the activation of TGF $\beta$  in the setting of biliary atresia may in part be mediated through both increased  $\alpha\text{v}\beta\text{8}$  expression and altered expression of MMP14. Further studies will be required to explore this in more depth.

### Concluding remarks

The molecular pathways that regulate TGF $\beta$  signalling are attractive targets for novel anti-fibrotic therapies. However, currently available methods of pan-TGF $\beta$  inhibition target all three mammalian isoforms and, because of the critical role of TGF $\beta$  in normal tissue homeostasis, this has led to serious concerns about potential side effects. For example, given its anti-proliferative effect on most epithelial cell types, there is a risk that TGF $\beta\text{1}$  inhibition could promote carcinogenesis. This is particularly relevant in advanced liver fibrosis, where most hepatocellular carcinomas originate from cirrhotic tissue. Additionally, because of the critical immunosuppressive role of TGF $\beta\text{1}$  (Shull et al. 1992; Kulkarni et al. 1993; Yaswen et al. 1996), pan-TGF $\beta$  blockade can lead to

excessive autoimmunity and inflammation, which could be highly deleterious in a fibrotic organ with limited functional reserve. Therefore, the inhibition of specific  $\alpha$ v integrin subunits might allow a more refined and targeted approach to TGF $\beta$  pathway inhibition, providing the desired anti-fibrotic effects but with fewer undesirable side effects.

In recent years, it has become apparent that  $\alpha$ v integrins play a key role in fibrosis in multiple organs. Abundant *in vivo* data are now available demonstrating critical regulatory roles for  $\alpha$ v integrins expressed on multiple 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, myofibroblasts and the cells of the immune system during the development and resolution of fibrosis. Strategies to manipulate  $\alpha$ v integrins, such as antibody blockade and small molecule inhibitors, will hopefully yield effective anti-fibrotic therapies in the future.

#### Compliance with ethical standards

**Conflict of interest** The authors declare there is no conflict of interest.

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