J. Cell. Mol. Med. Vol 16, No 3, 2012 pp. 445-455

αV integrins and TGF-β-induced EMT: a circle of regulation

Fahmy A. Mamuya, Melinda K. Duncan *

Department of Biological Sciences, University of Delaware, Newark, DE, USA

Received: March 22, 2011; Accepted: August 9, 2011

Abstract

Transforming growth factor-β (TGF-β) has roles in embryonic development, the prevention of inappropriate inflammation and tumour suppression. However, TGF-β signalling also regulates pathological epithelial-to-mesenchymal transition (EMT), inducing or progressing a number of diseases ranging from inflammatory disorders, to fibrosis and cancer. However, TGF-β signalling does not proceed linearly but rather induces a complex network of cascades that mutually influence each other and cross-talk with other pathways to successfully induce EMT. Particularly, there is substantial evidence for cross-talk between αV integrins and TGF-β during EMT, and anti-integrin therapeutics are under development as treatments for TGF-β-related disorders. However, TGF-β's complex signalling network makes the development of therapeutics to block TGF-β-mediated pathology challenging. Moreover, despite our current understanding of integrins and TGF-β function during EMT, the precise mechanism of their role during physiological *versus* pathological EMT is not fully understood. This review focuses on the circle of regulation between αV integrin and TGF-β signalling during TGF-β induced EMT, which pose as a significant driver to many known TGF-β-mediated disorders.

Keywords: EMT • TGF-β mediated disorders • αV integrins

EMT and pathogenesis: the good, the bad and the ugly

Epithelial-to-mesenchymal transition contributes to diverse developmental events including blastocyst implantation, gastrulation, generation of the neural crest, closure of the palate and normal wound healing [1–3]. Paradoxically, EMT also plays a role in pathological wound healing, tissue fibrosis and aspects of the EMT-like transitions that occur in many types of cancer [4, 5]. Morphologically, EMT is defined as the loss of epithelial characters such as apical–basolateral polarity, tight and adherens junctions and the ability to synthesize basement membranes. Concomitantly, these cells develop a fibroblastic morphology by rearranging their actin cytoskeleton, become migratory by forming filopodia and lamellopodia, interact with stromal extracellular matrices (ECM) due to changes in cell surface matrix receptors such as integrins, begin direct synthesis of stromal ECM and, in some cases, particularly in scar formation, become contractile myofibroblasts [3, 6, 7]. However, due to its functional diversity and complexity, scientists have long disagreed on a clear definition of EMT. A recent international meeting on EMT proposed to solve this dispute by classifying EMT into three different types: Type1, Type2 and Type3 (Fig. 1) [8, 9]. Type1 EMT occurs during the earliest stages of

*Correspondence to: Melinda K. DUNCAN, Department of Biological Sciences, Newark, DE 19716, USA.

Tel.: +1-(302)-831-0533
Fax: +1-(302)-831-2281
E-mail: duncanm@udel.edu

doi:10.1111/j.1582-4934.2011.01419.x

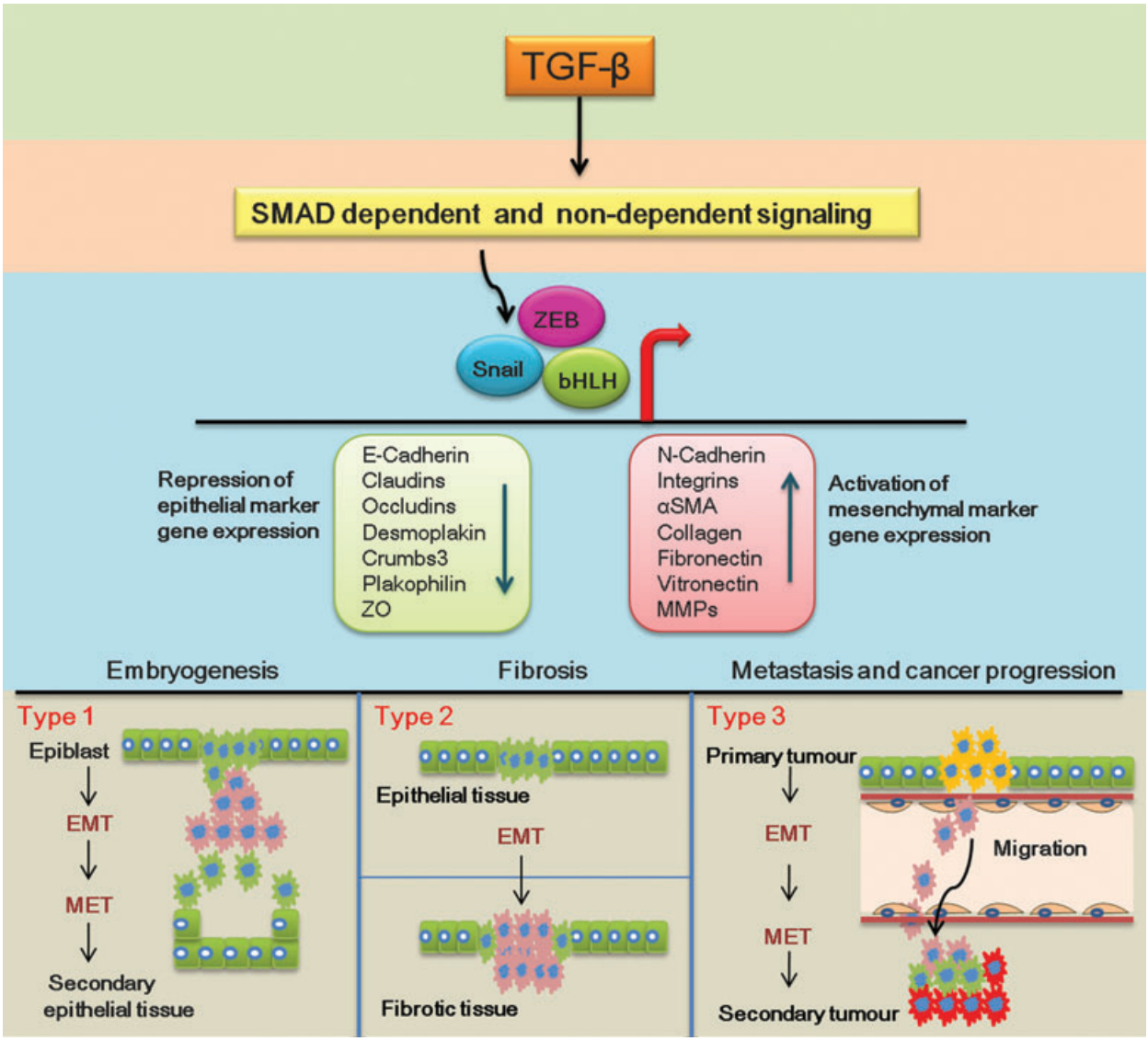

Fig. 1 Epithelial mesenchymal transition (EMT) occurs when epithelial cells lose their epithelial cell characteristics and become mesenchymal. Mesenchymal cells can return to an epithelial phenotype, a process called mesenchymal–epithelial transition (MET). Type1 EMT: During embryogenesis, the primitive epithelium (the epiblast) undergoes EMT forming primary mesenchyme that can migrate and undergo MET to form secondary epithelia that differentiate into new epithelial tissues. Type2 EMT: In mature or adult tissues, epithelial cells can also undergo EMT following local cellular disorganization caused by various stressors, inflammation or wounding but fail to undergo MET leading to fibroblast production and finally fibrosis. Type3 EMT: Epithelial cancer cells can undergo EMT to acquire a more migratory mesenchymal phenotype that allows them to invade secondary epithelia and proliferate as secondary tumours. During the process, migrating mesenchymal cells will have to intravasate, migrate through vasculature and extravasate to invade secondary tissue, undergo MET and proliferate forming secondary tumours. Green (epithelial cells), pink (mesenchymal), yellow (primary tumour), red (secondary tumour).

development, for example, during implantation and embryogenesis. Type2 EMT is defined as that which occurs in more mature epithelial tissues. In contrast to Type1 EMT, Type2 EMT can be triggered by inflammation or wound-healing responses and may lead to fibrosis. Finally, Type 3 EMT is the loss of epithelial and the gain of mesenchymal characters associated with cancer progression and metastasis.

TGF-β - a central player in EMT

Although several growth factors participate in EMT [10], Transforming growth factor-β is the most studied, with evidence suggesting that it can drive all three classes of EMT [11, 12]. Further, TGF-β also regulates a wide array of other cellular

processes including cell division, differentiation, motility, apoptosis and tumour suppression [13, 14]. There are three known isoforms of TGF-β in mammals, TGF-β_1, TGF-β_2 and TGF-β_3 [15]. The expression and function of all three isoforms varies dramatically among tissues and can even vary from species to species [16, 17]. Therefore, the mechanism by which TGF-β function is regulated and its signals transmitted within cells is very complex. TGF-β is synthesized within the secretory pathway as a precursor, which is cleaved to form the functional 25 kD TGF-β homodimer and the latency-associated peptide (LAP). LAP and TGF-β homodimer remain associated as a non-covalently bound complex known as the small latent complex (SLC). The SLC remains in the secretory pathway until it is bound by latent TGF-β-binding protein (LTBP) to form the large latent complex (LLC). The LLC is secreted and bound either covalently or non-covalently to the ECM [18, 19]. In most cases, the LLC will remain in the ECM until it is further processed to release active TGF-β [20]. Upon its release, the active TGF-β cytokine ignites TGF-β signalling by first binding to the type II TGF-β receptor, inducing a conformational change in its serine/threonine kinase domain and recruiting the type I TGF-β receptor to form the active receptor complex. Once the receptor complex is formed, the type II receptor kinase phosphorylates multiple serine and threonine residues in the TTSGSGSG sequence of the cytoplasmic GS region of the type I receptor, leading to the activation of TGF-β-induced signalling [21].

The best-studied pathway mediating TGF-β function is the SMA-Mothers against decapentaplegic family member dependent (canonical) pathway, which initiates when receptor-regulated SMAD 2 and/or 3 (r-SMAD2/3) is recruited to the activated TGF-β receptor by the SMAD anchor for receptor activation [22]. The type I TGF-β receptor then phosphorylates r-SMAD2/3 inducing conformational changes in the MH2 domain of r-SMAD2/3 and its subsequent dissociation from the receptor complex. Once phosphorylated and released, the phosphorylated r-SMAD2/3 attains a high affinity towards the co-SMAD (SMAD4) and binds to it [23]. The complex formed by r-SMAD2/3 and co-SMAD4 translocates to the nucleus where, in the case of EMT, it represses epithelial gene transcription while transcriptionally activating the expression of mesenchymal genes as well as other transcription factors capable of regulating EMT, such as members of Snail, ZEB and bHLH families [16, 24]. This canonical pathway can also induce EMT *via* cross-talk with other signalling pathways. For instance, SMAD-mediated TGF-β signalling can activate integrin-linked kinase (ILK), which allows ILK to phosphorylate GSK-3β and Akt family serine/threonine protein kinase (Akt), leading to β-catenin nuclear translocation and activation of other transcription factors, resulting in EMT of renal tubular epithelial cells [25, 26]. Transforming growth factor-β can also mediate EMT through its non-canonical (SMAD independent) pathways [27]. For instance, TGF-β-induced activation of Erk/MAP kinase, Rho GTPase and the PI3 kinase/Akt pathways can result in all three EMT types [28, 29]. This is an area of active investigation; more non-canonical pathways and additional levels of regulation are still being discovered [30].

Recently it was shown that TGF-β signalling can regulate the expression of microRNAs, which play a crucial role in regulating EMT [31]. MicroRNAs belonging to the miR-200 and miR-205 family can prevent EMT by down-regulating the EMT-associated transcription factors ZEB1 and SMAD interacting protein 1 (SIP1) [32]. Because miR-200 and miR-205 expression is reduced upon TGF-β stimulation, this suggests that TGF-β is a key regulator of the expression of microRNAs that block EMT [33]. It is likely that other microRNAs either synergize with or antagonize TGF-β signalling during TGF-β induced EMT, thus, investigating how TGF-β signalling regulates microRNA expression during EMT is important for the eventual development of therapeutics that target TGF-β function.

Changes in cell–cell/cell–ECM adhesion in EMT—the role of integrins during EMT

Interactions between cells and ECM convey micro-environmental cues that influence cell behaviour and function [34, 35]. Integrins are heterodimeric transmembrane proteins consisting of one α- and one β-subunit. Integrins facilitate interactions between cells and the ECM and play major roles in cell proliferation, differentiation, adhesion and migration [36]. At least 24 different integrin heterodimers exist, which recognize diverse components of the ECM including laminin, collagen, heparan sulfate proteoglycans, vitronectin, fibronectin, osteopontin, bone sialoprotein, thrombospondin, fibrinogen and tenascin [37, 38]. Further, changes in ECM composition, such as the increased expression of fibronectin, vitronectin and type I collagen seen during EMT, can switch integrins from an inactive low affinity to an active high-affinity ligand binding state [39, 40]. This can result in outside—in integrin signalling mediated *via* adaptor proteins that bind to the cytoplasmic tail of integrins such as ILK, paxillin, focal adhesion kinase (FAK) and five LIM domains containing focal adhesion protein (PINCH). Similarly, intracellular cues can also activate integrin-associated proteins affecting integrin affinity for extracellular ligands, a process known as inside-out signalling [41].

Thus, in the case of EMT, perturbations of the ECM microenvironment or intracellular cues can persuade integrins to dictate adhesion changes between cells and the ECM (and in some cases cells to cells), subsequently inducing disassembly of tight and adherens junctions, dissolution of desmosomes, actin reorganization and loss of epithelial apical–basal polarity, which are all associated with EMT. In extremis, these changes initiate focal adhesion complex formation leading to cell migration and invasion [42]. For example, β5 integrin regulates cell–cell/cell–ECM adhesion changes upon TGF-β signalling and β5 integrin depletion reduces the invasiveness of breast carcinoma cells by impairing the dissociation of tight junctions and/or reducing cell–ECM adhesion [43]. Further, β1 integrin engagement with collagen type I results in loss of E-cadherin and indirect up-regulation of N-cadherin, suggesting that integrin activation can directly result in EMT [44, 45]. Integrins can also facilitate changes in cell–ECM contact during EMT by binding to membrane Type-1 matrix metalloproteinase (MT1-MMP) to control their function [46].

TGF-β as a regulator of integrin expression during EMT

Many αV integrins, especially αVβ3, αVβ5 and αVβ6 are expressed at low levels in healthy epithelial tissues but up-regulate during both type2 and type3 EMT [47–49]. For example, αVβ6 integrin is highly up-regulated during pulmonary inflammation, fibrosis and cervical squamous cell carcinoma, where its increased expression correlates with poor prognosis [50, 51]. This implies that the TGF-β signalling that induces EMT also plays an essential role in the up-regulation of integrin expression. A recent study showed that TGF-β1 signalling during renal fibrosis enhances SMAD3 binding to the β1 integrin promoter, triggering an up-regulation of β1 integrin gene expression. Interestingly, knockdown or functional blockade of either β1 integrin or SMAD3, slowed the progression of renal fibrosis [52]. Another study showed that TGF-β1-induced signalling increased both β3 integrin subunit mRNA and protein levels as well as surface expression of αVβ3 in human lung fibroblasts *via* a β3 integrin, c-Src family of non-receptor tyrosine kinases (Src)- and p38 MAPK-dependent pathway [53].

TGF-β-mediated integrin up-regulation can also be indirect. TGF-β is a key regulator of ECM production, deposition and remodelling [54, 55], while integrin expression is partially regulated by the cellular microenvironment [40]. Thus, TGF-β-mediated up-regulation of ECM protein expression can indirectly stimulate integrin expression. For example, TGF-β-induced fibronectin expression in MRC-5 human myofibroblasts subsequently induces the expression of αVβ3, αVβ1 and α1β1 integrins [56]. TGF-β-induced signalling can also regulate integrin activation states by mediating the expression of integrin-associated adaptor proteins such as PINCH-1, which binds to ILK and plays an essential role in integrin signalling [57–59]. Further, TGF-β signalling can directly activate integrins by phosphorylating the cytoplasmic tail of the β integrin subunit. A recent study reports that TGF-β1 promotes vascular invasion by phosphorylating β1 integrin's threonine 788–789 *via* the TGF-β receptor, inducing inside-out signalling that ultimately drives hepatocellular carcinoma invasion [60]. Thus, by regulating integrin expression and function, TGF-β gains the ability to regulate cell biology in a tissue- and cell-specific fashion. However, in spite of the emerging understanding of how TGF-β signalling can regulate integrins during EMT progression, it is much less clear why these processes are tissue and cell specific.

αV integrins activate TGF-β

Transforming growth factor-β is secreted in a latent form which must be activated extracellularly to efficiently trigger receptor-mediated TGF-β signalling. Diverse activation mechanisms have been demonstrated which allow for regulation of TGF-β function in different cellular/tissue contexts [61]. Among the three TGF-β isoforms, only the LAPs of TGF-β1 and TGF-β3 contain an integrin binding motif, arginine–glycine–aspartic acid (RGD). Notably, many αV integrins including αVβ1, αVβ3, αVβ5, αVβ6 and αVβ8 can interact with this RGD sequence resulting in activation of TGF-β1 and TGF-β3 [50, 62–65]. Not surprisingly, mice with a non-functional variant of the RGD sequence in their TGF-β1 LAP express normal levels of latent TGF-β1, but display features similar to that of TGF-β1 knockouts [50, 66]. Moreover, mice lacking αVβ6 and αVβ8, phenocopy TGF-β1 and TGF-β3 knockouts confirming the importance of αV integrins in TGF-β1 and TGF-β3 activation [67]. Furthermore, antibody-mediated blockade of αV integrin function, particularly αVβ6, down-regulates the TGF-β-induced EMT and inflammation known to causes fibrosis, metastasis and cancer [68–71]. Two different models of how αV integrins can activate TGF-β are proposed, both of which have significant experimental support. It appears that the choice of TGF-β activation mechanism is cell-type specific and is influenced by whether the appropriate integrin is expressed and the specific cell physiological context. However, it is not known whether the use of a particular TGF-β-activating mechanism influences the resulting TGF-β signalling.

Conformational change activation mechanism

Upon binding to the LAP of TGF-β1 and TGF-β3, αV integrins can exert adhesion-mediated cell forces inducing conformational changes of the LAP, which results in structural deformation of the latent complex and liberation of active TGF-β [50]. Drugs which block myofibroblast contraction such as ML-7, blebbistatin, cytochalasin D and the α-smooth muscle actin (α-SMA) contraction inhibitor SMA-FP, also inhibited activation of TGF-β1 by contraction-inducing drugs [72, 73]. Analogously, studies that inhibited αVβ5 integrin function by forming αvβ5–Thy1 complexes also inhibit contraction-induced latent TGF-β1 activation [74]. Because TGF-β signalling induces a contractile cytoskeleton along with α-SMA expression during EMT, this can result in further TGF-β activation [75]. Although this direct activation does not require proteolysis of either the LAP or LTBP, it does require a mechanically stiff ECM and contractile cytoskeleton which provides sufficient forces to liberate TGF-β from its latent complex [20, 76].

Conformational change activation mechanism with proteolysis

Not all integrin interactions with the LAP directly activate TGF-β, particularly in the absence of a stiff ECM. Moreover, active TGF-β must be physically proximal to a type II TGF-β receptor for activation of TGF-β signalling. Notably, several MMPs such MMP-9 and MMP-2 can proteolytically cleave the LAP and/or LTBP to liberate TGF-β from its ECM bound stores [77, 78]. Further, αV integrins

can interact with MMPs, such as MMP2 and MMP9, to tether them to the cell surface [79, 80]. Thus, in certain physiological contexts, αV integrins can simultaneously promote proximity of MMPs to the LAP and sequester the LLC close to the type II TGF-β receptor. Depending on the cell/tissue type, this mechanism can function without significant cell traction [76, 81], although a contractile cytoskeleton and a mechanically resistant ECM can also participate [73, 76].

Overall, αV integrins can activate TGF-β, which induces EMT, which then in turn creates an ideal microenvironment for further unregulated TGF-β activation resulting in pathological EMT. Recently, the known complexity of this mechanism has been increased by a report showing that αV integrins also interact with the TGF-β co-receptor, endoglin, and this interaction can enhance αV integrin mediated TGF-β activation [82]. However, the extent to which cell type, magnitude of the ECM's mechanical resistance, the relative proximity of latent TGF-β to proteinases and other known TGF-β activators such as pH, thrombospondin-1 and reactive oxygen species [20] play a role, remains unclear.

TGF-β and integrin signalling cross-talk during EMT

Inactive integrins are not usually bound to the ECM or tightly linked to the actin cytoskeleton, instead, they are scattered diffusely over the cell surface. However, upon their binding to ECM, a purposeful association with the actin cytoskeleton is induced, leading to integrin clustering into focal adhesion complexes, which, depending on the properties of the ECM, will instigate integrin signalling [83, 84]. This signal is propagated to the intracellular integrin associated adaptor proteins such as ILK, Src, PTKs and FAK; which subsequently activate other downstream players such as MAPK, Ras/Rho, small GTPases, PI3K and AKT [85]. On the other hand, canonical TGF-β signalling initiates upon receptor activation and SMAD phosphorylation but can indirectly activate MAPK and PI3K pathways as well [86]. In addition, non-canonical TGF-β signalling can directly activate MAPK, PI3, Ras/Rho and small GTPase pathways [30]. Therefore, cross-talk between TGF-β and integrin signalling can occur downstream of initial receptor activation and regulate various cellular processes [87], which may override the tumour suppressing function of TGF-β [88–90] or lead to other TGF-β-associated disorders [91]. TGF-β signalling can also result in either direct or indirect phosphorylation of integrin adaptor proteins, while integrins can associate with receptor tyrosine kinases (RTKs) to activate TGF-β signalling resulting in tumour invasion and metastasis [49]. Further, TGF-β1 signalling can induce tyrosine phosphorylation of FAK including its autophosphorylation site, Tyr-397, leading to inside-out integrin signalling that subsequently induces myofibroblast differentiation in fibrotic diseases [85]. TGF-β1 induced signalling can also phosphorylate other proteins such as the p85 subunit of PI3K, and serine 473 of Akt resulting in the up-regulation of αV integrin expression that is necessary for the migration of human chondrosarcoma cells [86].

On the other hand, integrin association with RTKs may activate the integrin—receptor-tyrosine-kinase signalling that is necessary for development, tumour invasion and metastasis. For example, TGF-β signalling can lead to the transcriptional up-regulation of αVβ3 integrin expression, which then enhances the functional interaction between the type II TGF-β receptor and αVβ3 integrins; a situation that potentiates the proliferative effects of TGF-β-induced EMT in human lung fibroblasts [92]. Type II TGF-β receptor and αVβ3 integrin interactions can also auto-stimulate type II TGF-β receptor phosphorylation on its tyrosine residues *via* Src; initiating a TGF-β signalling induced stimulation of MAPKs that leads to EMT and invasion in epithelial cells [93]. Overall, more potential points of cross-talk between integrin and TGF-β signalling are being discovered, yet even now, the mechanistic differences that allow TGF-β to suppress tumourigenic processes in healthy tissue and promote tumour formation by cancer cells still remain unclear.

Disease perspectives

Active TGF-β has a high affinity for its receptors, so, in most cases, a released/active TGF-β ligand will initiate a TGF-β signalling cascade as long as TGF-β receptors are within reach [94]. Because different cellular functions require distinct levels of TGF-β signalling [76], tight regulation of latent TGF-β activation is necessary to prevent diverse diseases including inflammation, autoimmune disorders, fibrosis, cancer and cataract [95–99]. Conversely, inadequate levels of active TGF-β due to mutation of either the TGF-β genes or those for TGF-β activators can lead to pathology. For instance, TGF-β1-deficient mice exhibit a multifocal, mixed inflammatory cell response and tissue necrosis, leading to organ failure and death 20 days after birth [100, 101]. In humans, inadequate TGF-β signalling can result in various disorders including brain haemorrhage and immune system-associated disorders [102, 103]. Notably, restoring normal TGF-β signalling and/or inhibiting its inappropriate expression in experimental animals reverses some TGF-β-associated pathologies and stands as promising therapeutic approach (Table 1). TGF-β-induced signalling is also known to destabilize E-cadherin-mediated cell–cell adhesion during EMT [104]. Fascinatingly, a study in renal fibrosis revealed that bone morphogenic protein (BMP)-7 can reverse TGF-β1-induced EMT by restoring E-cadherin expression hence halting EMT progression. These results suggest that cross-talk between BMP-7 and TGF-β is essential to regulate pathological EMT [105]. αV integrins can activate TGF-β by binding to its LAP and evidence of such activation has been linked in EMT progression. Therefore, blocking the undesirable activities of αV integrins without interfering with their beneficial functions could impede EMT progression during cancer, wound healing and fibrosis.

Table 1 Attempts to target αV integrin function as a therapeutic strategy to treat TGF-β–associated disorders

Integrin	Disorder	Experimental findings
αVβ3	1. Atherosclerosis	1. Blockade of αVβ3 reduced neointima formation by reducing TGF-β activity [113]
αVβ5	2. Rheumatoid arthritis	2. Integrin αVβ3 as a target for the treatment of rheumatoid arthritis and related rheumatic diseases [114]
	3. Systemic sclerosis	3. Increased expression of integrin αVβ5 contributes to the establishment of autocrine TGF-β signalling in scleroderma fibroblasts [115]
αVβ6	1. Inflammation	1. αVβ6 protects against inflammatory periodontal disease through activation of TGF-β [98]
	2. Carcinoma	2. Blockade of integrin αVβ6 inhibits tumour progression *in vivo* by a TGF-β regulated mechanism [70]
	3. Fibrosis	3. Inhibitors of αVβ6 integrin or TGF-β down-regulate fibrosis following acute or ongoing pulmonary, biliary injury, renal injury [68, 69]
	4. Cataracts	4. αVβ6 was hypothesized to be the main activator of TGF-β1 in the lens capsule and represents a possible target for the prevention of posterior capsular opacification [99]
αVβ8	1. Immune dysfunction	1. αVβ8-mediated TGF-β activation by dendritic cells is essential to prevent inflammatory bowel disease and autoimmunity [103]
	2. COPD	2. αVβ8 integrin-mediated TGF-β activation amplifies pathologic epithelial-mesenchymal in chronic obstructive pulmonary disease patients [116]
	3. Brain haemorrhage	3. αVβ8 acts as a central regulator of brain vessel homeostasis through its regulation of TGF-β activation [102]

Integrin antagonists show clinical promise for the treatment of TGF-β-induced EMT-associated disorders such as inflammation, fibrosis and cancer [106]. Most of the therapeutic approaches currently under investigation target integrin function using anti-integrin agents including both naturally occurring and engineered peptides that can mimic their RGD ligand, or antibodies that can act as integrin antagonists [107–109]. For example, clinical administration of a peptide antagonist of the αVβ3 receptor successfully inhibits pathological angiogenesis seen in cancer, proliferative retinopathy, rheumatoid arthritis and psoriasis [110, 111]. Similarly, TGF-β-mediated enhancement of glioma cell migration *via* the up-regulation of αVβ3 integrin expression is abrogated by echistatin, a Arg-Gly-Asp (RGD) containing snake venom which is a potent antagonist of αVβ3 integrin [112].

Several integrin-targeted therapies are in clinical development for the treatment of cancer [117]. For instance, Cilengitide or EMD12197 (Merck KGaA, Darmstadt, Germany) is a small cyclic RGD designed peptide that selectively and competitively antagonizes ligand binding to αVβ3 and αVβ5 [118], which is being evaluated in a phase III clinical study for treatment of glioblastoma [119]. A number of monoclonal antibodies are also in clinical development. CNTO 95 is a fully humanized monoclonal antibody targeting αV integrin, which shows anti-tumour and anti-metastatic activity in animal models and is in a Phase I clinical trial for the treatment of solid tumours [120, 121]. Similarly, Vitaxin, also known as MEDI-522 or Abegrin, is also a humanized monoclonal antibody that can block the interaction of αVβ3 with various ligands such as osteopontin, latent TGF-β and vitronectin [114]. Vitaxin is currently in clinical trials for the treatment of stage IV metastatic melanoma and androgen-independent prostate cancer [122]. Notably, MedImmune Inc. ended advanced human testing of Vitaxin to treat rheumatoid arthritis and psoriasis in 2004 because it failed to show clinical benefits in initial studies [123]. More recently, it was shown that pre-treatment of osteoclasts with macrophage colony stimulating factor (M-CSF), which is known to activate αVβ3, enhanced Vitaxin's inhibitory effect. Furthermore, the PI3-kinase inhibitor wortmannin abolished M-CSF's effects on the action of Vitaxin suggesting that Vitaxin's inhibitory effects require an activated form of αVβ3 integrin and that PI3-kinase signalling is involved in the process [124]. On the other hand, numerous studies have shown that PI3K-Akt signalling is involved in TGF-β-induced EMT and cell migration [125, 126]. This exemplifies how understanding the cross-talk between αV integrins and TGF-β signalling can enhance the therapeutic potential of not only Vitaxin, but other integrin antagonists as well, to make better and more successful therapeutics.

Completing the circle

Integrins and their signalling can regulate EMT by both perturbing cell adhesion and stimulating EMT associated gene expression. In addition, integrins containing the αV subunit can activate latent TGF-β to result in TGF-β-induced EMT. Concomitantly, TGF-β

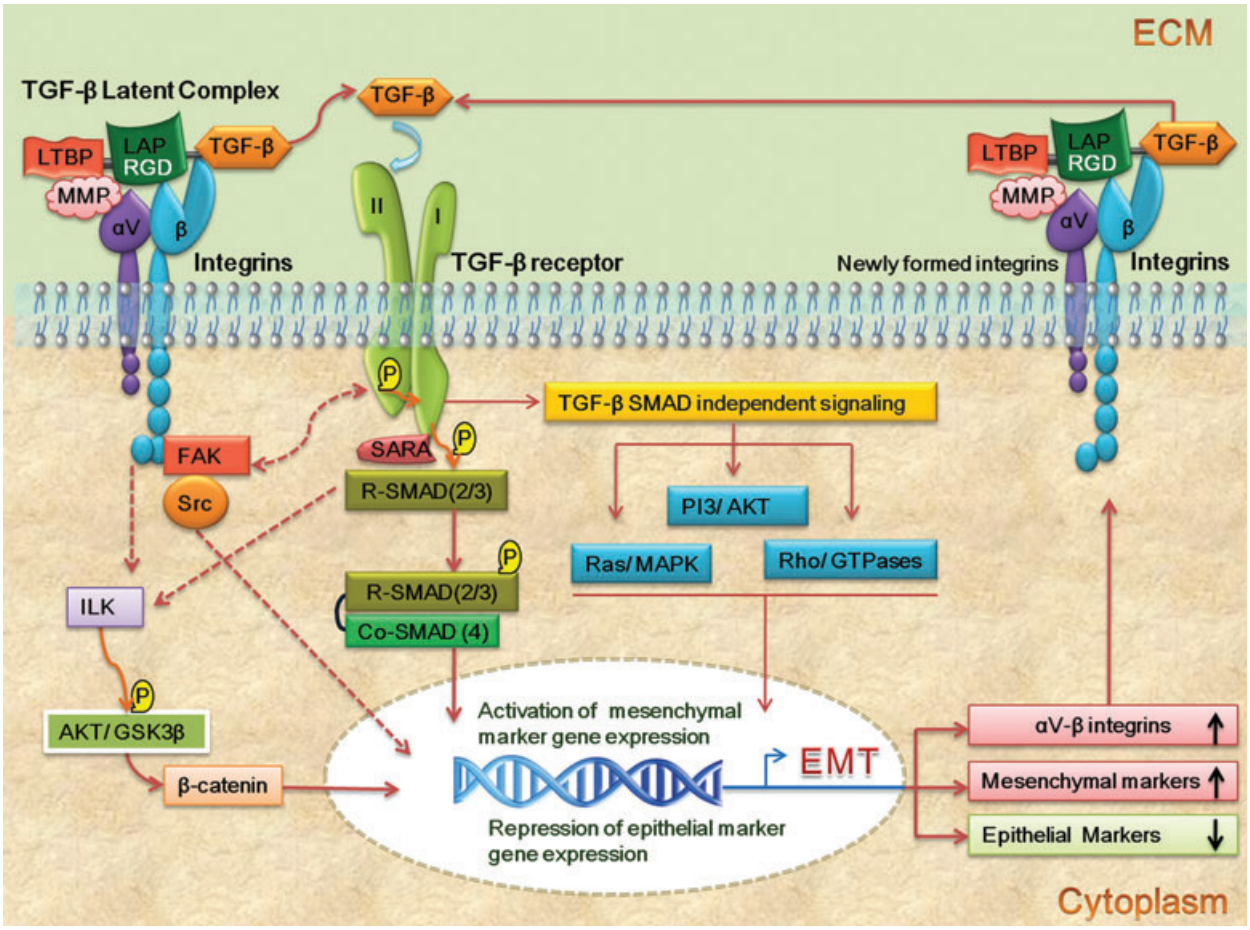

Fig. 2 αV integrins recognize an RGD motif present in the LAP of TGF-β. This binding induces either adhesion-mediated cell forces and/or brings latent TGF-β into the proximity of MMPs, which consequently lead to the liberation/activation of the TGF-β homodimer from its latent complex. Upon activation, the TGF-β homodimer will bind to the Type II TGF-β receptor initiating TGF-β-Smad signalling, which upregulates the expression of αV integrins in addition to that of other EMT markers. These newly formed integrins can liberate more TGF-β from its latent complex, sustaining and reinforcing TGF-β induced EMT progression. This cooperative feed forward loop between αV integrins and TGF-β can lead to the unregulated TGF-β signalling responsible for a number of TGF-β–associated disorders.

signalling can activate integrins and also up-regulate integrin expression. TGF-β signalling can also induce a contractile cytoskeleton and a stiff cellular microenvironment to further facilitate latent TGF-β activation by αV integrins. This altogether creates a feed-forward circle of cross-regulation between αV integrins and TGF-β (Fig. 2) that can drive the EMT responsible for TGF-β-associated disorders ranging from inflammation, fibrosis and cancer. Because experimental approaches that interfere with αV integrin function block TGF-β-mediated disease pathogenesis in experimental models, elucidation of the molecular participants in both physiological and pathological EMT as well as differences in their regulation, may lead to selective therapies for TGF-β-associated disorders.

Acknowledgements

This work was supported by National Eye Institute Grant No. EY015279 and its training supplement No. EY015279 S1. Special thanks to all members of Dr Melinda Duncan's laboratory group for critical reading of the manuscript.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

References

1. **Duband JL, Thiery JP.** Appearance and distribution of fibronectin during chick embryo gastrulation and neurulation. *Develop Biol*. 1982; 94: 337–50.
2. **Vicovac L, Aplin JD.** Epithelial-mesenchymal transition during trophoblast differentiation. *Acta Anat (Basel)*. 1996; 156: 202–16.
3. **Hay ED.** An overview of epithelio-mesenchymal transformations. *Acta Anat*. 1995; 154: 8–20.
4. **Chaffer CL, Thompson EW, Williams ED.** Mesenchymal to epithelial transition in development and disease. *Cells Tissues Organs*. 2007; 185: 7–19.
5. **Jose Miguel L-N, Nieto MA.** Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. *EMBO Mol Med*. 2009; 1: 303–14.
6. **Kalluri R, Neilson EG.** Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest*. 2003; 112: 1776–84.
7. **Thiery JP, Acloque H, Huang RY, *et al.*** Epithelial-mesenchymal transitions in development and disease. *Cell*. 2009 139: 871–90.
8. **Kalluri R, Weinberg RA.** The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009; 119: 1420–8.
9. **Zeisberg M, Neilson EG.** Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest*. 2009; 119: 1429–37.
10. **Strutz F, Zeisberg M, Ziyadeh FN, *et al.*** Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation. *Kidney Int*. 2002; 61: 1714–28.
11. **Nawshad A, LaGamba D, Polad A, *et al.*** Transforming growth factor-β^2 signaling during epithelial-mesenchymal transformation: implications for embryogenesis and tumour metastasis. *Cells Tissues Organs*. 2005; 179: 11–23.
12. **Bhowmick NA, Ghiassi M, Bakin A, *et al.*** Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Mol Biol Cell*. 2001; 12: 27–36.
13. **Taipale J, Saharinen J, Keski-Oja J.** Extracellular matrix-associated transforming growth factor-beta: role in cancer cell growth and invasion. *Adv Cancer Res*. 1998; 75: 87–134.
14. **Yue J, Mulder KM.** Transforming growth factor-[beta] signal transduction in epithelial cells. *Pharmacol Therap*. 2001; 91: 1–34.
15. **Derynck R, Lindquist PB, Lee A, *et al.*** A new type of transforming growth factor-beta, TGF-beta 3. *EMBO J*. 1988; 7: 3737–43.
16. **Massagué J.** TGF-β in cancer. *Cell*. 2008; 134: 215–30.
17. **Massague J, Cheifetz S, Laiho M, *et al.*** Transforming growth factor-beta. *Cancer Surv*. 1992; 12: 81–103.
18. **Nunes I, Gleizes P-E, Metz CN, *et al.*** Latent transforming growth factor-β binding protein domains involved in activation and transglutaminase-dependent cross-linking of latent transforming growth factor-β. *J Cell Biol*. 1997; 136: 1151–63.
19. **Chen Q, Sivakumar P, Barley C, *et al.*** Potential role for heparan sulfate proteoglycans in regulation of transforming growth factor-β (TGF-β) by modulating assembly of latent TGF-β-binding protein-1. *J Biol Chem*. 2007; 282: 26418–30.
20. **Annes JP, Munger JS, Rifkin DB.** Making sense of latent TGF{beta} activation. *J Cell Sci*. 2003; 116: 217–24.
21. **Shi Y, Massagué J.** Mechanisms of TGF-[beta] signaling from cell membrane to the nucleus. *Cell*. 2003; 113: 685–700.
22. **Wu G, Chen Y-G, Ozdamar B, *et al.*** Structural basis of Smad2 recognition by the smad anchor for receptor activation. *Science*. 2000; 287: 92–7.
23. **Souchelnytskyi S, Rönnstrand L, Heldin C-H, *et al.*** Phosphorylation of Smad signaling proteins by receptor serine/threonine kinases: protein kinase protocols. *Methods Mol Biol*. 2001; 124: 107–20.
24. **Peinado H, Olmeda D, Cano A.** Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer*. 2007; 7: 415–28.
25. **Li Y, Tan X, Dai C, *et al.*** Inhibition of integrin-linked kinase attenuates renal interstitial fibrosis. *J Am Soc Nephrol*. 2009; 20: 1907–18.
26. **Delcommenne M, Tan C, Gray V, *et al.*** Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. *Proc Natl Acad Sci USA*. 1998; 95: 11211–6.
27. **Derynck R, Zhang YE.** Smad-dependent and Smad-independent pathways in TGF-[beta] family signalling. *Nature*. 2003; 425: 577–84.
28. **Zavadil J, Bitzer M, Liang D, *et al.*** Genetic programs of epithelial cell plasticity directed by transforming growth factor-beta. *Proc Natl Acad Sci USA*. 2001; 98: 6686–91.
29. **Xu J, Lamouille S, Derynck R.** TGF-[beta]-induced epithelial to mesenchymal transition. *Cell Res*. 2009; 19: 156–72.
30. **Zhang YE.** Non-Smad pathways in TGF-beta signaling. *Cell Res*. 2009; 19: 128–39.
31. **Zavadil J, Narasimhan M, Blumenberg M, *et al.*** Transforming growth factor-β and microRNA:mRNA regulatory networks in epithelial plasticity. *Cells Tissues Organs*. 2007; 185: 157–61.
32. **Gregory PA, Bert AG, Paterson EL, *et al.*** The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol*. 2008; 10: 593–601.
33. **Pandit KV, Corcoran D, Yousef H, *et al.*** Inhibition and role of let-7d in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2010; 182: 220–9.
34. **Slavkin HC.** Combinatorial process for extracellular matrix influences on gene expression: a hypothesis. *J Craniofac Genet Dev Biol*. 1982; 2: 179–89.
35. **Lukashev ME, Werb Z.** ECM signalling: orchestrating cell behaviour and misbehaviour. *Trends Cell Biol*. 1998; 8: 437–41.
36. **Richard O H.** Integrins: a family of cell surface receptors. *Cell*. 1987; 48: 549–54.
37. **Ruoslahti E.** RGD and other recognition sequences for integrins. *Ann Rev Cell Develop Biol*. 1996; 12: 697–715.
38. **Desgrosellier JS, Cheresh DA.** Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer*. 2010; 10: 9–22.
39. **Imamichi Y, Menke A.** Signaling pathways involved in collagen-induced disruption of the E-cadherin complex during epithelial-mesenchymal transition. *Cells Tissues Organs*. 2007; 185: 180–90.
40. **Boudreau NJ, Jones PL.** Extracellular matrix and integrin signalling: the shape of things to come. *Biochem J*. 1999; 339: 481–8.
41. **Anthis NJ, Campbell ID.** The tail of integrin activation. *Trends Biochem Sci*. 2011; 36: 191–8.
42. **Yilmaz M, Christofori G.** EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev*. 2009; 28: 15–33.
43. **Bianchi A, Gervasi ME, Bakin A.** Role of β5-integrin in epithelial-mesenchymal transition in response to TGF-β. *Cell Cycle* 2010; 9: 1647–59.

44. **Koenig A, Mueller C, Hasel C, *et al.*** Collagen type I induces disruption of E-cadherin–mediated cell-cell contacts and promotes proliferation of pancreatic carcinoma cells. *Cancer Res*. 2006; 66: 4662–71.
45. **Shintani Y, Fukumoto Y, Chaika N, *et al.*** Collagen I mediated up-regulation of N-cadherin requires cooperative signals from integrins and discoidin domain receptor 1. *J Cell Biol*. 2008; 180: 1277–89.
46. **Gonzalo P, Moreno V, Gálvez BG, *et al.*** MT1-MMP and integrins: hand-to-hand in cell communication. *BioFactors*. 2010; 36: 248–54.
47. **Breuss JM, Gallo J, DeLisser HM, *et al.*** Expression of the beta 6 integrin subunit in development, neoplasia and tissue repair suggests a role in epithelial remodeling. *J Cell Sci*. 1995; 108: 2241–51.
48. **Breuss JM, Gillett N, Lu L, *et al.*** Restricted distribution of integrin beta 6 mRNA in primate epithelial tissues. *J Histochem Cytochem*. 1993; 41: 1521–7.
49. **Guo W, Giancotti FG.** Integrin signalling during tumour progression. *Nat Rev Mol Cell Biol*. 2004; 5: 816–26.
50. **Munger JS, Huang X, Kawakatsu H, *et al.*** A mechanism for regulating pulmonary inflammation and fibrosis: the integrin [alpha]v[beta]6 binds and activates latent TGF [beta]1. *Cell*. 1999; 96: 319–28.
51. **Hazelbag S, Kenter GG, Gorter A, *et al.*** Overexpression of the αvβ6 integrin in cervical squamous cell carcinoma is a prognostic factor for decreased survival. *J Pathol*. 2007; 212: 316–24.
52. **Yeh Y-C, Wei W-C, Wang Y-K, *et al.*** Transforming growth factor-[beta]1 induces Smad3-dependent [beta]1 integrin gene expression in epithelial-to-mesenchymal transition during chronic tubulointerstitial fibrosis. *Am J Pathol*. 2010; 177: 1743–54.
53. **Pechkovsky DV, Scaffidi AK, Hackett TL, *et al.*** Transforming growth factor beta 1induces αVβ3 integrin expression in human lung fibroblasts *via* a β3 integrin-, c-Src-, and p38 MAPK-dependent pathway. *J Biol Chem*. 2008; 283: 12898–908.
54. **Schiller M, Javelaud D, Mauviel A.** TGF-[beta]-induced SMAD signaling and gene regulation: consequences for extracellular matrix remodeling and wound healing. *J Dermatol Sci*. 2004; 35: 83–92.
55. **Verrecchia F, Mauviel A.** Transforming growth factor-[bgr] signaling through the smad pathway: role in extracellular matrix gene expression and regulation. 2002; 118: 211–5.
56. **Honda E, Yoshida K, Munakata H.** Transforming growth factor-beta; upregulates the expression of integrin and related proteins in MRC-5 human myofibroblasts. *Tohoku J Exp Med*. 2010; 220: 319–27.
57. **Wu C.** Integrin-linked kinase and PINCH: partners in regulation of cell-extracellular matrix interaction and signal transduction. *J Cell Sci*. 1999; 112: 4485–9.
58. **Wu C.** PINCH, N(i)ck and the ILK: network wiring at cell-matrix adhesions. *Trends Cell Biol*. 2005; 15: 460–6.
59. **Li Y, Dai C, Wu C, *et al.*** PINCH-1 promotes tubular epithelial-to-mesenchymal transition by interacting with integrin-linked kinase. *J Am Soc Nephrol*. 2007; 18: 2534–43.
60. **Fransvea E, Mazzocca A, Antonaci S, *et al.*** Targeting transforming growth factor (TGF)-βRI inhibits activation of β1 integrin and blocks vascular invasion in hepatocellular carcinoma. *Hepatology*. 2009; 49: 839–50.
61. **Miyazono K, Heldin CH.** Latent forms of TGF-beta: molecular structure and mechanisms of activation. *Ciba Found Sympos*. 1991; 157: 81–9; discussion 9–92.
62. **Mu D, Cambier S, Fjellbirkeland L, *et al.*** The integrin αvβ8 mediates epithelial homeostasis through MT1-MMP–dependent activation of TGF-β1. *J Cell Biol*. 2002; 157: 493–507.
63. **Annes JP, Rifkin DB, Munger JS.** The integrin [alpha]V[beta]6 binds and activates latent TGF[beta]3. *FEBS Lett*. 2002; 511: 65–8.
64. **Annes JP, Chen Y, Munger JS, *et al.*** Integrin {alpha}V{beta}6-mediated activation of latent TGF-{beta} requires the latent TGF-{beta} binding protein-1. *J Cell Biol*. 2004; 165: 723–34.
65. **Ludbrook SB, Barry ST, Delves CJ, *et al.*** The integrin alphavbeta3 is a receptor for the latency-associated peptides of transforming growth factors beta1 and beta3. *Biochem J*. 2003; 369: 311–8.
66. **Yang Z, Mu Z, Dabovic B, *et al.*** Absence of integrin-mediated TGF-beta1 activation *in vivo* recapitulates the phenotype of TGF-beta1-null mice. *J Cell Biol*. 2007; 176: 787–93.
67. **Aluwihare P, Mu Z, Zhao Z, *et al.*** Mice that lack activity of αvβ6- and αvβ8-integrins reproduce the abnormalities of Tgfb1- and Tgfb3-null mice. *J Cell Sci*. 2009; 122: 227–32.
68. **Wang B, Dolinski BM, Kikuchi N, *et al.*** Role of αvβ6 integrin in acute biliary fibrosis. *Hepatology*. 2007; 46: 1404–12.
69. **Horan GS, Wood S, Ona V, *et al.*** Partial inhibition of integrin {alpha}v 6 prevents pulmonary fibrosis without exacerbating inflammation. *Am J Respir Crit Care Med*. 2008; 177: 56–65.
70. **Koopman Van Aarsen LA, Leone DR, Ho S, *et al.*** Antibody-mediated blockade of integrin αvβ6 inhibits tumour progression *in vivo* by a transforming growth factor-β–regulated mechanism. *Cancer Res*. 2008; 68: 561–70.
71. **Bates RC, Bellovin DI, Brown C, *et al.*** Transcriptional activation of integrin β6 during the epithelial-mesenchymal transition defines a novel prognostic indicator of aggressive colon carcinoma. *J Clin Invest*. 2005; 115: 339–47.
72. **Hinz B, Gabbiani G, Chaponnier C.** The NH2-terminal peptide of alpha smooth muscle actin inhibits force generation by the myofibroblast *in vitro* and *in vivo*. *J Cell Biol*. 2002; 157: 657–63.
73. **Wipff P-J, Rifkin DB, Meister J-J, *et al.*** Myofibroblast contraction activates latent TGF-β1 from the extracellular matrix. *J Cell Biol*. 2007; 179: 1311–23.
74. **Zhou Y, Hagood JS, Lu B, *et al.*** Thy-1-integrin alpha(v)beta(5) interactions inhibit lung fibroblast contraction-induced latent transforming growth factor-beta activation and myofibroblast differentiation. *J Biol Chem*. 2010; 285: 22382–93.
75. **Kurosaka D, Kato K, Nagamoto T, *et al.*** Growth factors influence contractility and alpha-smooth muscle actin expression in bovine lens epithelial cells. *Invest Ophthalmol Visual Sci*. 1995; 36: 1701–8.
76. **Wipff P-J, Hinz B.** Integrins and the activation of latent transforming growth factor [beta]1—an intimate relationship. *Eur J Cell Biol*. 2008; 87: 601–15.
77. **Yu Q, Stamenkovic I.** Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumour invasion and angiogenesis. *Genes Develop*. 2000; 14: 163–76.
78. **Dallas SL, Rosser JL, Mundy GR, *et al.*** Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. *J Biol Chem*. 2002; 277: 21352–60.
89. **Brooks PC, Stromblad S, Sanders LC, *et al.*** Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. *Cell*. 1996; 85: 683–93.
80. **Rolli M, Fransvea E, Pilch J, *et al.*** Activated integrin αvβ3 cooperates with metalloproteinase MMP-9 in regulating migration of metastatic breast cancer cells. *Proc Natl Acad Sci USA*. 2003; 100: 9482–7.

81. **Yu Q, Stamenkovic I.** Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-Î² and promotes tumour invasion and angiogenesis. *Genes Develop*. 2000; 14: 163–76.
82. **Rivera LB, Brekken RA.** SPARC promotes pericyte recruitment *via* inhibition of endoglin-dependent TGF-β1 activity. *J Cell Biol*. 2011; 193: 1305–19.
83. **Schoenwaelder SM, Burridge K.** Bidirectional signaling between the cytoskeleton and integrins. *Curr Opin Cell Biol*. 1999; 11: 274–86.
84. **Berrier AL, Yamada KM.** Cell–matrix adhesion. *J Cell Physiol*. 2007; 213: 565–73.
85. **Dedhar S.** Integrins and signal transduction. *Curr Opin Hematol*. 1999; 6: 37–43.
86. **Guo X, Wang X-F.** Signaling cross-talk between TGF-[beta]/BMP and other pathways. *Cell Res*. 2009; 19: 71–88.
87. **Cary LA, Han DC, Guan JL.** Integrin-mediated signal transduction pathways. *Histol Histopathol*. 1999; 14: 1001–9.
88. **Wendt MK, Schiemann WP.** Therapeutic targeting of the focal adhesion complex prevents oncogenic TGF-beta signaling and metastasis. *Breast Cancer Res*. 2009; 11: R68.
89. **Sieg DJ, Hauck CR, Ilic D, *et al.*** FAK integrates growth-factor and integrin signals to promote cell migration. *Nat Cell Biol*. 2000; 2: 249–56.
90. **Playford MP, Schaller MD.** The interplay between Src and integrins in normal and tumour biology. *Oncogene*. 2004; 23: 7928–46.
91. **Worthington JJ, Klementowicz JE, Travis MA.** TGFbeta: a sleeping giant awoken by integrins. *Trends Biochem Sci*. 2011; 36: 47–54.
92. **Scaffidi AK, Petrovic N, Moodley YP, *et al.*** Alpha(v)beta(3) integrin interacts with the transforming growth factor beta (TGFbeta) type II receptor to potentiate the proliferative effects of TGFbeta1 in living human lung fibroblasts. *J Biol Chem*. 2004; 279: 37726–33.
93. **Galliher A, Schiemann W.** Beta3 integrin and Src facilitate transforming growth factor-beta mediated induction of epithelial-mesenchymal transition in mammary epithelial cells. *Breast Cancer Res*. 2006; 8: R42.
94. **Massague J.** Subunit structure of a high-affinity receptor for type beta-transforming growth factor. Evidence for a disulfide-linked glycosylated receptor complex. *J Biol Chem*. 1985; 260: 7059–66.
95. **Roberts AB, Wakefield LM.** The two faces of transforming growth factor beta in carcinogenesis. *Proc Natl Acad Sci USA*. 2003; 100: 8621–3.
96. **Derynck R, Akhurst RJ, Balmain A.** TGF-[beta] signaling in tumour suppression and cancer progression. *Nat Genet*. 2001; 29: 117–29.
97. **Irina GL, Nevins WT, Natalia N, *et al.*** Regulation of pulmonary inflammation and fibrosis through expression of integrins alphaVbeta3 and alphaVbeta5 on pulmonary T lymphocytes. *Arthritis Rheumat*. 2009; 60: 1530–9.
98. **Ghannad F, Nica D, Garcia Fulle MI, *et al.*** Absence of {alpha}v{beta}6 integrin is linked to initiation and progression of periodontal disease. *Am J Pathol*. 2008; 172: 1271–86.
99. **Ulrike S, Stefan P, Afschin S, *et al.*** Upregulation of αVβ6 integrin, a potent TGF-β1 activator, and posterior capsule opacification. *J. Cataract Refract Surgery*. 2005; 31: 595–606.
100. **Sterner-Kock A, Thorey IS, Koli K, *et al.*** Disruption of the gene encoding the latent transforming growth factor-β binding protein 4 (LTBP-4) causes abnormal lung development, cardiomyopathy, and colorectal cancer. *Genes Develop*. 2002; 16: 2264–73.
101. **Shull MM, Ormsby I, Kier AB, *et al.*** Targeted disruption of the mouse transforming growth factor-[beta]1 gene results in multifocal inflammatory disease. *Nature*. 1992; 359: 693–9.
102. **Cambier S, Stephanie G, Dezhi M, *et al.*** Integrin alpha (v)beta8-mediated activation of transforming growth factor-beta by perivascular astrocytes: an angiogenic control switch. *Am J Pathol*. 2005; 166: 1883–94.
103. **Travis MA, Reizis B, Melton AC, *et al.*** Loss of integrin αVβ8 on dendritic cells causes autoimmunity and colitis in mice. *Nature*. 2007; 449: 361–5.
104. **Vogelmann R, Nguyen-Tat MD, Giehl K, *et al.*** TGFbeta-induced downregulation of E-cadherin-based cell-cell adhesion depends on PI3-kinase and PTEN. *J Cell Sci*. 2005; 118: 4901–12.
105. **Zeisberg M, Bottiglio C, Kumar N, *et al.*** Bone morphogenic protein-7 inhibits progression of chronic renal fibrosis associated with two genetic mouse models. *Am J Physiol Renal Physiol*. 2003; 285: F1060–7.
106. **Wang Z, Chui W-K, Ho PC.** Integrin targeted drug and gene delivery. *Expert Opin Drug Deliv*. 2010; 7: 159–71.
107. **Chernousov MA, Carey DJ.** [alpha]V [beta]8 integrin is a Schwann cell receptor for fibrin. *Exp Cell Res*. 2003; 291: 514–24.
108. **Palmade F S-CO, Regnouf de Vains JB, Coquelet C, *et al.*** Inhibition of cell adhesion to lens capsule by LCM 1910, an RGD-derived peptide. *J Ocul Pharmacol*. 1994; 10: 623–32.
109. **Oharazawa HIN, Ohara K, Reddy VN.** Inhibitory effects of Arg-Gly-Asp (RGD) peptide on cell attachment and migration in a human lens epithelial cell line. *Ophthalmic Res*. 2005; 37: 191–6. doi: 10.1159/000086595
110. **Del Gatto A, Zaccaro L, Grieco P, *et al.*** Novel and selective αvβ3 receptor peptide antagonist: design, synthesis, and biological behavior. *J Med Chem*. 2006; 49: 3416–20.
111. **D'Andrea LD, Del Gatto A, Pedone C, *et al.*** Peptide-based molecules in angiogenesis. *Chem Biol Drug Design*. 2006; 67: 115–26.
112. **Platten M, Wick W, Wild-Bode C, *et al.*** Transforming growth factors [beta]1 (TGF-[beta]1) and TGF-[beta]2 promote glioma cell migration *via* up-regulation of [alpha]V[beta]3 integrin expression. *Biochem Biophys Res Commun*. 2000; 268: 607–11.
113. **Coleman KR, Braden GA, Willingham MC, *et al.*** Vitaxin, a humanized monoclonal antibody to the vitronectin receptor ({alpha}vfl3), reduces neointimal hyperplasia and total vessel area after balloon injury in hypercholesterolemic rabbits. *Circ Res*. 1999; 84: 1268–76.
114. **Wilder RL.** Integrin alpha V beta 3 as a target for treatment of rheumatoid arthritis and related rheumatic diseases. *Ann Rheum Dis*. 2002; 61: ii96–9.
115. **Asano Y, Ihn H, Yamane K, *et al.*** Increased expression of integrin Î±vÎ²3 contributes to the establishment of autocrine TGF-Î² signaling in scleroderma fibroblasts. *J Immunol*. 2005; 175: 7708–18.
116. **Araya J, Cambier S, Markovics JA, *et al.*** Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD patients. *J Clin Invest*. 2007; 117: 3551–62.
117. **Nemeth JA, Nakada MT, Trikha M, *et al.*** Alpha-v integrins as therapeutic targets in oncology. *Cancer Invest*. 2007; 25: 632–46.
118. **Nisato R, Tille J-C, Jonczyk A, *et al.*** αvβ3 and αvβ5 integrin antagonists

inhibit angiogenesis *in vitro*. *Angiogenesis*. 2003; 6: 105–19.

119. **Tabatabai G, Weller M, Nabors B, *et al.*** Targeting integrins in malignant glioma. *Targeted Oncol*. 2010; 5: 175–81.
120. **Chen Q, Manning C, Millar H, *et al.*** CNTO 95, a fully human anti αv integrin antibody, inhibits cell signaling, migration, invasion, and spontaneous metastasis of human breast cancer cells. *Clin Exp Metastasis*. 2008; 25: 139–48.
121. **Mullamitha SA, Ton NC, Parker GJM, *et al.*** Phase I evaluation of a fully human anti-αv integrin monoclonal antibody (CNTO 95) in patients with advanced solid tumours. *Clinical Cancer Res*. 2007; 13: 2128–35.
122. **Tucker GC.** Integrins: molecular targets in cancer therapy. *Curr Oncol Rep*. 2006; 8: 96–103.
123. **Rosenwald MS.** MedImmune ends some vitaxin testing. The Washington Post; 2004.
124. **Gramoun A, Shorey S, Bashutski JD, *et al.*** Effects of Vitaxin®, a novel therapeutic in trial for metastatic bone tumours, on osteoclast functions *in vitro*. *J Cell Biochem*. 2007; 102: 341–52.
125. **Bakin AV, Tomlinson AK, Bhowmick NA, *et al.*** Phosphatidylinositol 3-kinase function is required for transforming growth factor beta-mediated epithelial to mesenchymal transition and cell migration. *J Biol Chem*. 2000; 275: 36803–10.
126. **Runyan CE, Schnaper HW, Poncelet AC.** The phosphatidylinositol 3-kinase/Akt pathway enhances Smad3-stimulated mesangial cell collagen I expression in response to transforming growth factor-beta1. *J Biol Chem*. 2004; 279: 2632–9.