In: Myofibroblasts
Editor: Analise Martinez
ISBN: 978-1-63485-743-7


***Chapter 1***

# INTEGRINS: FORM AND ROLE IN MYOFIBROBLAST DIFFERENTIATION AND FUNCTION

***Qiang Ma*, Jie Dong and Bridget Hindman***
Receptor Biology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV, US

## ABSTRACT

Myofibroblasts play a major role in connective tissue remodeling by synthesizing the extracellular matrix (ECM) and by exerting contraction, both of which depend on complex and specific cell-matrix and cell-cell communications. Integrins are a major family of transmembrane receptors that directly link the intracellular cytoskeleton dynamics to extracellular structures via adhesions in all metazoans. Human integrins are heterodimeric proteins composed of one of the 18 α and one of the eight β subunits to form 24 distinct isoforms. Each integrin has unique, sometimes overlapping, tissue expression, ligands, and functions, and each is capable of cross-interacting with numerous signaling pathways

* Corresponding author: Qiang Ma. Receptor Biology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 1095 Willowdale Road, Morgantown, WV 26505, US. Email: qam1@cdc.gov.

through their intracellular tails and extracellular receptor domains. These structural and functional features enable integrins to transmit both chemical and mechanical signals bidirectionally between cells and the dynamic pericellular environment, which is critical for both physiologic functions, such as embryonic morphogenesis and wound healing, and disease processes, such as organ fibrosis and cancer. Several forms of integrins have been identified in myofibroblasts that seemingly form a core molecular pathway to regulate the differentiation and function of myofibroblasts in physiology and disease. In this chapter, we discuss major integrins that have an established role in the formation, function, and turnover of myofibroblasts associated with tissue remodeling. Particular attention is given to β1- and αv-containing integrins relating to their role and mode of action in myofibroblast activation during wound healing and organ fibrosis.

**Keywords**: myofibroblast, integrin, wound healing, fibrosis, ECM, α-SMA, TGF-β, adhesion

## INTRODUCTION

Myofibroblasts are a heterogeneous group of cells that are distinguished by their simultaneous presentation of the extracellular matrix (ECM)-synthesizing capability of fibroblasts and the cytoskeletal contractile characteristics of smooth muscle cells [1, 2]. Cells with such dual phenotypes are found at sites of tissue injury and in organ fibrosis, where they function both as the primary ECM-secreting cells to produce and remodel the ECM and as the major contractile cells responsible for the contractility of granulation and scar tissues [3, 4]. Myofibroblasts are also found in carcinomas where they play an important role in creating the tumor microenvironment necessary for tumor initiation, progression, invasion, and metastasis [5-7]. Moreover, fibroblastic cells with myofibroblast features can be identified in normal organs and tissues, such as the lung septa, hepatic perisinusoidal space, and bone-marrow stroma [8-10]. As such, it has been increasingly recognized that myofibroblasts contribute importantly to the neo-synthesis and remodeling of connective tissues with ramifications for a wide range of physiologic and disease processes [2, 11].

The function and mode of action of myofibroblasts have been incompletely understood. Studies on dermal wound healing have shed some light onto the role and dynamic fate of myofibroblasts during physiological

and pathological tissue repair. The early phase response following wounding starts with blood clotting that initially seals the wound. This is followed by inflammatory infiltration, reepithelialization, basement membrane regeneration, and angiogenesis, each with a distinctive temporal-spacial pattern [12]. The formation of a granulation tissue begins simultaneously with the reepithelialization to replace the provisional wound matrix. The granulation tissue is essentially composed of small vessels, fibroblasts, myofibroblasts, and a variable amount of inflammatory cells. Among the cells, myofibroblasts are recognized as a major contributor to the *de novo* formation and contraction of the granulation matrix. As the reepithelialization approaches completion, myofibroblasts undergo apoptosis causing granulation to evolve into a poorly cellularized scar, which would enhance the strength of the repaired wound [13].

In pathological fibrosis, scarring does not occur and granulation tissue develops into a hypertrophic fibrotic mass with many myofibroblasts and an inappropriate production of ECM, leading to the deformation and malfunction of the tissue or organ [11]. The failure of myofibroblasts to disappear via appotosis, as occurs during the late stage of normal wound healing, is thought to account for the persistence of these cells in fibrosing tissues. This general scheme of fibrosis formation appears to be applicable to fibrosis of internal organs following various types of injury. Chronic wound healing is another type of pathologic tissue response to injury in which the formation of granulation tissue is reduced and the wound fails to heal over a prolonged period of time. An abberant myofibroblastic response may contribute to the development of chronic wound healing [14].

In these models of wound healing, the formation, function, and fate of myofibroblasts illustrate complex and dynamic interactions of the cell with its surrounding matrix and neighboring, and sometimes distant, cells. Moreover, these interactions are highly regulated in a temporal-, spacial-, and context-dependent manner by numerous signals. The nature of the signals and the molecular underpinnings of these interactions and regulations remain unclear to a large extent. Nonetheless, it is conceivable that such interactions and regulations would require the accurate sensing of both chemical and mechanical signals, bidirectional signal transduction, and the coupling of cytoskeletal contractile dynamics to alterations in the ECM.

Integrins are a family of transmembrane receptors expressed in all metazoans [15]. Mammalian integrins are heterodimers of non-covalently associated $\alpha$ and $\beta$ subunits. Integrin isoforms with different combinations of $\alpha$ and $\beta$ polypeptides are expressed in a tissue-, cell type-, development-, and

physiologic and disease context-dependent manner [16, 17]. Integrins do not possess enzymatic activities and thus they are not considered as classical cell surface signaling receptors. Instead, integrin signaling depends largely on their allosteric activities: their direct link to both cellular cytoskeletal contractile actins and the pericellular environment; their presence as a core component of adhesions; and their ability to recruit enzymes, signaling proteins, and ligands both inside and outside cells to result in the formation of supracomplex, integrin-based cell adhesions [15]. Combined, these functions enable integrins to directly sense both chemical and tensional signals, cross-talk with numerous and diverse signaling pathways, and mediate the contraction of cells that controls cell shape and movement as well as ECM remodeling. Research in recent years has revealed that a list of isoforms of integrins are expressed in myofibroblasts and their related cells, and these isoforms have significant impacts on multiple aspects of myofibroblast functions and dynamics in physiology and disease, which is the focus of discussion of this chapter.

## FORM, FUNCTION AND STRUCTURE OF INTEGRINS

Mammalian species have 18 α and eight β integrin subunits that heterodimerize to form 24 distinct isoforms in humans and presumably many mammalian species [15]. These subunits are restricted to metazoans, though some structural pieces appeared early in evolution, such as the von Willebrand factor A domain (vWFA) found in prokaryotes [18]. Based on the phylogenetic relationship of their α subunits, ligand specificity, and tissue expression, integrin isoforms can be separated into five groups or subfamilies. The first group consists of isoforms α5β1, α8β1, αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, and αIIbβ3, all of which recognize the tripeptide sequence arginine-glycine-aspartic acid (RGD) of ligands, such as the ECM proteins fibronectin, vitronectin, and thrombospondins, and the plasma protein fibrinogen, and are thus termed RGD receptors. The laminin receptors comprise the 2nd group including α3β1, α6β1, α7β1, and α6β4 integrins, which mediates cell adhesion to basement membrane laminins. These first two groups of integrins appear to be more ancient in origin than the remaining isoforms, which may reflect the early evolution of integrins to allow cell-matrix adhesion intrinsic to metazoa and asymmetric interactions of cells with the basal lamina in diploblastic organisms. The 3rd group of integrins includes a set of collagen receptors, such as α1β1, α2β1, α10β1, and α11β1, which have an extra

inserted domain (also called vWFA domain) in the α subunit (αI, αA, or I/A). These integrins bind various types of collagens and thus are collagen receptors. The two integrins containing α4 or α9—i.e., α4β1 and α9β1—are expressed in chordates similarly to collagen receptors, but they don't contain an I/A domain and don't bind collagens. Instead, they recognize ECM proteins, such as fibronectin, in an RGD-independent manner, as well as immunoglobulin (Ig)-superfamily cell surface counterreceptors, such as vascular cell adhesion molecule-1 (VCAM-1). The 5$^{th}$ group is a set of leukocyte-specific receptors including αDβ2, αLβ2, αMβ2, αXβ2, αEβ7, and α4β7, which are expressed in white blood cells and recognize Ig-superfamily counterreceptors, such as intercellular adhesion molecules (ICAMs) and complement component 3b (C3b), to mediate heterotypic cell-cell adhesion.

The functions of integrin isoforms are complex. Some subunits, such as β1 and αv, are produced in a larger excess and are more promiscuous than others. These subunits form heterodimers with multiple α or β subunits, perform a range of functions, and, in the case of β1, can be hijacked by other biological systems, such as bacteria, in their interaction with host cells. Most subunits appear to have distinctive functions, which is most clearly shown by the phenotypes of knockout mice of individual integrin genes [15, 17]. Many isoforms also exhibit unique substrate specficity and tissue expression, which is partly reflected in their subfamily classification discussed above. To date, the genes encoding all 18 α and eight β subunits have been knocked out in mice, which reveals distinct phenotypes for individual knockouts, ranging from a complete blockade of preimplementation development (β1) and embryonic or perinatal lethality (α3, α6, α8, β4, β8), to major developmental defects (α4, α5, αv, β8), defects in specific functions such as homeostasis (αIIb, α2, β3), leukocyte performance (αL, αE, αM, β2, β7), bone and cartilage formation/remodeling (β3, α10), inflammation (β6), angiogenesis (α1, β3), or growth (dwarfism; α10, α11), and no apparent abnormality (β5). Some major integrin knockout mice and their phenotypes are summarized in Table 1 along with references. Tissue-specific or induced mouse gene knockouts were used to characterize the adult phenotypes and specific functions in cases where the global knockout of an integrin gene is embryonic or perinatal lethal. These studies have revealed broader and more detailed functions of integrins in specific tissues, cell types, and physiologic and disease processes, such as wound healing and organ fibrosis, as discussed in more detail in later sections (Table 1). Integrins can also be hijacked by invading microbes. For instance, enteropathogenic *Yersinia* species express a

surface protein that may directly bind to an integrin, as exemplified by the binding of the *Yersinia* protein invasin to α5β1 of an epithelial cell; alternatively, the protein may bind to fibronectin within tissue to bridge between the bacterium and an integrin of a target cell, as exemplified by the binding of the *Yersinia* protein YadA to α5β1. In either scenario, the binding to integrin triggers integrin-mediated phagocytosis, which enables the bacterium to gain entry into the host cell [19, 20]. In aggregate, integrins and their ligands play critical roles in mammalian development, tissue homeostasis, immune responses, leukocyte trafficking, organ function, and the development of numerous human diseases including fibrosis, cancer, and infection.

The multifarious and complex functions performed by integrins have a clear root in their structure [16, 21]. The α and β subunits of integrins do not share a sequence homology to each other, but have a similar overall organization constructed from several domains with flexible linkers between them. Each polypepetide typically contains ~1000 (for α subunits) or 750 (for β subunits) amino acid residues that form a short unstructured tail at the carboxyl terminal end, a single transmembrane helix domain, and a large, amino-terminal, ectodomain structure (Figure 1) [22, 23]. The ectodomain consists of an extracellular ligand-binding head to provide the ligand- and intersubunit-binding interface and a multi-domain "leg" to support the "head" in each subunit. The α subunit ligand-binding head is a β-propeller structure with 7 repeats forming the blades. The collagen receptor integrins have an I/A domain of ~200 residues inserted between the 2$^{nd}$ and 3$^{rd}$ propeller repeats, forming a metal ion-dependent adhesion site (MIDAS) important for ligand binding. The α subunit leg consists of a thigh domain and 2 calf domains. The β subunit ectodomain contains 4 epidermal growth factor (EGF) repeats, a hybrid domain (meaning split in sequence), an I-like domain (βI), and a plexin-sempahorin-integrin (PSI) domain. The βI domain also contains a MIDAS site for ligand binding similarly to αI.

The overall topology of unliganded integrins adopts a V-shape conformation in which the legs are severely bent and the head domains are closely juxtaposed to the membrane-proximal portions of the legs (Figure 1B). This bent conformation represents the physiological low-affinity state. Priming and ligand binding are associated with a large-scale global conformational rearrangement in which the bent integrin extends with a switchblade-like motion [22, 23]. This extended conformation is associated with affinity modulation and ligand binding. These structural features of integrins bring

together a platform for their regulated activity, such as ligand binding, outside-in and inside-out signal transduction, cross-talk with other signaling pathways, and coupling to cytoskeletal contractile actins.

**Table 1. Integrin Isoform with a Role in Myofibroblast Biology**

| Integrin Isoform | Classification; Typical Ligand | Expression; Cellular Function | Phenotype of Mouse KO; Link to human disease | Reference |
|---|---|---|---|---|
| α1β1 | COL receptor*; Col I, III, IV, LM-111. | ECs, FBs, MFBs, monocytes/macrophages; Negative feed back regulation of COL synthesis. MFB differentiation. | α1 KO: viable, defects in collagen synthesis, tumor angiogenesis, skin hardening. Increased COL expression during granulation tissue development in wound healing. β1 KO: embryonic lethal. β1 fibroblast-specific KO: delayed wound healing and resistance to scleroderma. | [24] [25, 26] [27, 28] |
| α2β1 | COL receptor; Col I and III, LM-332, TN-C, MMP-1, and CCN1. | PLTs, KCs, ECs, FBs; Regulates COL polymerization by FBs, KC migration, PLT adhesion to COL, and VEGF-driven angiogenesis. | α2 KO: viable, defects in PLT adhesion, mammary gland branching, and kidney development. Increased wound angiogenesis. | [29, 30] |
| α3β1 | Laminin receptor; LM-332, other LMs. | KCs, ECs, FBs; Regulates re-epithelialization, angiogenesis, and TGF-β1-driven responses. | α3 KO: perinatal lethal, defects in kidney and lung development, skin blistering. α3 keratinocyte-specific KO: impaired wound angiogenesis and re-epithelialization; Interstitial lung disease, nephrotic syndrome, epidermolysis bullosa. | [31] [32] |
| α4β1 | The α4/α9 group; FN, OPN, VCAM, EMILIN1. | Leukocytes, FBs, ECs; Interacts with EMILIN1. Regulates FB proliferation and TGF-β1 processing. | α4 KO: embryonic lethal, defects in heart, placenta, and hematopoietic development. | [33] |

**Table 1. (Continued)**

| Integrin Isoform | Classification; Typical Ligand | Expression; Cellular Function | Phenotype of Mouse KO; Link to human disease | Reference |
|---|---|---|---|---|
| α5β1 | RGD receptor; FN, CCN2, CCN3. | PLTs, ECs, leukocytes, FBs, KCs; Promotes KC migration. | α5 KO: embryonic lethal, defects in neural crest cell survival and mesoderm formation. | [34] |
| α6β1 | Laminin receptor; LMs, TSPs, CCN1, CCN2, CCN3. | PLTs, ECs, leukocytes, FBs; Regulates angiogenesis, MFB senescence, and fibrosis. | α6 KO: perinatal lethal, defects in cortex organization, skin blistering; Junctional epidermolysis bullosa. | [35] |
| α8β1 | RGD receptor; FN, VN, TN-C, latent TGF-β1. | MFBs; Contributes to fibrotic responses. | α8 KO: perinatal lethal, defects in kidney and inner ear development. | [36] |
| α9β1 | The α4/α9 group; FN, TN-C, OPN, ADAMs, VCAM, VEGFs, factor XIII, EMILIN1. | KCs, FBs, neutrophils, ECs; Regulates KC and FB growth, neutrophil chemotaxis, EC migration, and angiogenesis. | α9 KO: perinatal lethal, defects in lymphatic development, congenital chylothorax; Congenital chylothorax. | [37] |
| α10β1 | COL receptor; COLs. | FBs; Regulates FB adhesion to COLs. | α10 KO: viable, dwarfism with mild chondrodysplasia. | [38] |
| α11β1 | COL receptor; COLs. | FBs; Regulates MFB differentiation, FB adhesion to COLs, and COL reorganization. | α11 KO: viable, dwarfism with increased mortality, defect in periodontal ligament development. | [39] |
| αvβ1 | RGD receptor; FN, VN, OPN, latent TGF-β1. | KCs, ECs; Regulates KC adhesion during re-epithelialization and MFB differentiation. | αv KO: embryonic or perinatal lethal, defects in placenta formation and CNS development, cleft palate. αv myofibroblast-specific KO: resistance to induced fibrosis in liver, lungs, and kidneys. | [40] [41] |

| Integrin Isoform | Classification; Typical Ligand | Expression; Cellular Function | Phenotype of Mouse KO; Link to human disease | Reference |
|---|---|---|---|---|
| αvβ3 | RGD receptor; FN, VN, fibrinogen, TN-C, OPN, CCN1, CCN2, CCN3, latent TGF-β1. | ECs, PLTs, FBs, macrophages; Regulates neoangiogenesis, fibrin structure, EC adhesion, and FB proliferation. | β3 KO: viable, PLT abnormalities, bone defects; Glanzmann's thrombasthenia, thrombocytopenia. | [42] |
| αvβ5 | RGD receptor; VN, OPN, latent TGF-β1, CCN1, CCN3, VEGF. | ECs, FBs, KCs; Regulates FB transformation to MFB and FB migration through interactions with CCN1. | β5 KO: viable, no immediately observed defects. Wound healing is normal. | [43] |
| αvβ6 | RGD receptor; FN, VN, TN-C, TGF-β1 and –β3, chromogranin A. | KCs; Regulates inflammation, KC proliferation, and granulation tissue remodeling. | β6 KO: viable, defect in TGF-β activation, inflammation in skin and airways, impaired lung fibrosis; Asthma. | [44] |
| αvβ8 | RGD receptor; FN, latent TGF-β1, COL IV, LMs. | Dendritic cells, FBs, ECs; Regulates inflammation via TGF-β activation. | β8 KO: embryonic or perinatal lethal, defects in placenta formation and CNS development, cleft palate. | [45] |

* Abbreviations used Table 1: ADAM, a disintegrin and metalloproteinase; CCN, Cyr61-CTGF-Nov; Col, collagen; EDA, extra domain A; EC, endocyte; EGFR, epidermal growth factor receptor; FB, fibroblast; FN, fibronectin; ICAM, intercellular adhesion molecule; KC, keratinocyte; KO, knockout; LM, laminin; MMP, matrix metalloproteinase; OPN, osteopontin; PLT, platelet; TGF, transforming growth factor; TN, tenascin; TSP, thrombospondin; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VN, vitronectin.

## INTEGRINS AND MYOFIBROBLASTS IN WOUND HEALING

The functions of integrins at the cellular level can be separated into two groups, those related to their role as mechanical links in cell adhesion and migration and those related to signal sensing and transduction, both of which are essential components of the functions of fibroblasts and myofibroblasts. Accordingly, a list of integrins have been found to be expressed in fibroblasts,

myofibroblasts, and their related cells—i.e., progenitor cells such as pericytes that transdifferentiate into fibroblasts and myofibroblasts, and regulator cells such as keratinocytes that carry out reepithelialization and regulate myofibroblast differentiation underneath the new epiderm during wound healing. Many of these integrins have major impacts on myofibroblast biology and function (Table 1).

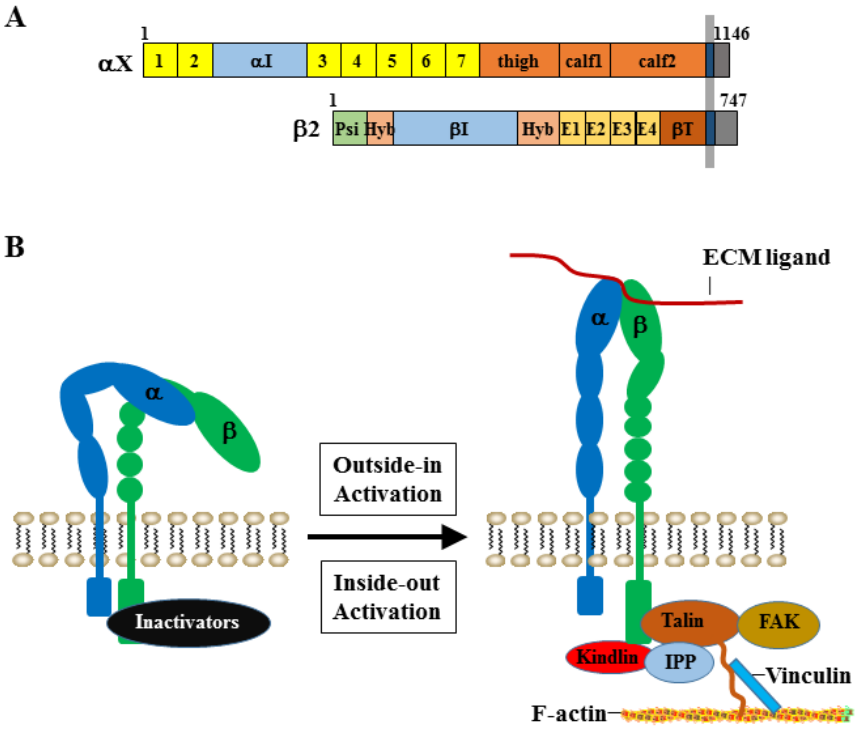


Figure 1. Integrin structure and activation. (A) Domain structure of αXβ2 [23]. (B) The bent, inactive and the extended, activated states of integrin. Inactivator proteins associate with integrin tail(s) to contribute to integrin inhibition. Inside-out signaling provokes the stepwise recruitment of multiple cytoplasmic proteins to integrin tails, including Talin, FAK, IPP, and Kindlin, which causes the straightening of integrin ectodomains to increase the affinity for ECM ligands. In a reciprocal manner, outside-in signaling triggered by ligand binding modulates intracellular signaling. The intracelluar integrin complex connects to cytoskeletal F-actin via the Talin carboxyl tail and Vinculin.

Animal studies with global or conditional knockout of individual integrin genes in mice provided the *in vivo* identification of integrin functions relating

to fibroblastic responses in physiologic and disease models. It is noted that *in vivo* animal studies often do not readily distinguish between fibroblasts and myofibroblasts, which is in part owing to the technical difficulty and a lack of specific markers in identifying myofibroblasts in tissues. For this reason and for the fact that certain mesenchymal functions during wound healing and fibrosis are shared between fibroblasts and myofibroblasts, some of the discussions below include both cell types. This ambiguity is less observed in cellular and molecular studies with cultured cells, where integrin isoforms are often identified with distinctive roles in specific myofibroblast functions and dynamics. It is also worth noting that the overall phenotype of a given integrin knockout may not be severe for a complex biological process such as wound healing and fibrosis, even though *in vitro* studies may have suggested a significant outcome if the function of the integrin isoform is compromised. This discrepancy between *in vivo* and *in vitro* findings on a specific integrin isoform is likely to be due to compensatory mechanisms provided by other integrins with overlapping functions and tissue expression. Given the wide range of functions of both integrins and fibroblast/myofibroblasts, we will use wound healing (see below) and organ fibrosis (see the following section) to illustrate the role of integrins in fibroblast and myofibroblast functions in physiology and disease.

Integrins regulate wound healing in many aspects including clotting, directional epithelial migration, epithelial cell proliferation, granulation tissue formation and remodeling, angiogenesis, and scar formation. Our discussion below is limited to processes that directly involve fibroblasts and myofibroblasts.

Upon wounding, activated fibroblasts (and precursor cells) migrate from adjacent subepithelial tissues into the wound bed wherein they differentiate into myofibroblasts to synthesize granulation tissue matrix and remodel it to a normal or scar tissue. This granulation tissue mixture of fibroblasts and myofibroblasts express many integrins, mainly the β1 and αv-containing integrins, such as α1β1, α2β1, α3β1, α5β1, α11β1, αvβ1, αvβ3, and αvβ5 [46]. These integrins mediate the binding of the cells to ECM molecules as well as their migration, contraction, secretion, and turnover during normal wound healing, and may contribute to their dysfunction during pathologic wound healing.

In the initial phase of wound healing, integrin α5β1 was found to mediate the adhesion of activated fibroblasts to the provisional matrix, a molecular step necessary for fibroblasts to migrate into the wound; this α5β1-dependent process was enhanced by addition of dermatopontin and tenascin-C [47, 48].

On the other hand, integrin αvβ3 appears to play an inhibitory role in fibroblast migration into the wound clot, as loss of αvβ3 function in mice (β3 knockout) leads to enhanced dermal fibroblast infiltration into the wound and faster epidermal wound healing [49].

The expression of α-smooth muscle actin (α-SMA)—a major marker and a major component of stress fibers of myofibroblasts—and the differentiation of fibroblasts into myofibroblasts become major events 1 week after wounding. Both β1 and αv integrins have been shown to play critical roles in myofibroblast differentiation. Knockout of β1 in mice is embryonic lethal [25, 26], whereas the fibroblast-specific deletion of β1 in mice permits the study of adult phenotypes to provide significant insights into myofibroblast function and regulation during wound healing [27]. Phenotypically, the mice exhibit delayed cutaneous wound closure and less granulation tissue formation with reduced expression of α-SMA and reduced production of new ECM. The primary dermal fibroblasts isolated from this β1-deficient mouse strain show reduced expression of α-SMA, collagen I, and CCN2 (i.e., connective tissue growth factor or CTGF), reduced adhesion to the ECM, reduced activation of latent TGF-β (transforming growth factor β) when cultured in a stressed collagen matrix, and ultimately, failure of differentiation of these cells into myofibroblasts. The phenotypes can be alleviated by administration of active TGF-β to the mice *in vivo*, thus establishing that integrin β1 is essential for normal wound healing and myofibroblast differentiation in a TGF-β-dependent manner [27]. Additionally, inhibition of α9β1 by a specific antibody decreased granulation tissue formation in a mouse model of skin wound healing [50]; whereas, induction of the collagen receptor α11β1 by mechanical strains promoted myofibroblast differentiation [51].

The role of αv integrins in myofibroblast differentiation relevant to wound healing was assessed in an *in vitro* study [52]. Blockade of the αv and/or β1 integrins by specific antibodies prevented the TGF-β1-induced myofibroblast differentiation, as assessed by α-SMA expression and collagen gel contraction by three human fibroblast cell lines. Inhibition of myofibroblast differentiation was further confirmed by antibody blockade of αvβ5 and αvβ3 in a cell type-dependent manner. On the other hand, increased expression of αvβ5 promoted myofibroblast differentiation by increasing the recruitment of the latent TGF-β complex onto the cell surface for activation via RGD recognition [53].

During granulation tissue remodeling, the tissue tension increases as fine collagen fibrils become thick collagen fibers, which appears to be regulated by

α2β1 expressed in myofibroblasts of the early granulation tissue. The resulting rigid matrix induces a switch of integrin expression from α2β1 to αvβ3 that may play a role in the late stage matrix remodeling [54]. A critical event in the late stage wound healing is the senescence of myofibroblasts, which may represent a programmed wound healing response that functions as a self-limiting mechanism for fibrogenesis. Myofibroblast senescence in skin wound healing is triggered by a dynamically expressed matricellular protein, CCN1 (i.e., cysteine-rich angiogenic inducer 61 or CYR61), which acts through integrin α6β1-mediated induction of oxidative stress [55]. However, how myofibroblasts become resistant to senescence and apoptosis and whether their persistent presence is sufficient to account for the occurrence of fibrosis instead of scarring during late stage wound healing remains uncertain.

Wound healing involves complex interactions among different cell types, some of which are regulated by integrins. For instance, αvβ6 is expressed in epithelial cells but regulates inflammation, ECM deposition, and myofibroblast differentiation in the granulation tissue underneath the epithelium, which may involve the activation and release of latent TGF-β1 by the epithelial cells locally [56]. The formation of a new basement membrane during wound healing is another example of close interactions between keratinocytes and fibroblasts because the basement membrane is synthesized jointly by the two types of cells. There is also a collaborative interaction between pericytes and endothelial cells for the assembly of vascular basement membranes during wound angiogenesis. In both scenarios, integrins appear to play a role.

Aberrant integrin signaling may contribute to the development of chronic wound healing, leading to a defective ECM that fails to support the reepithelialization and angiogenesis. For instance, defective integrin activation and cross-signaling in myofibroblasts would result in a diminished responsiveness of the cells to the stimulatory action of TGF-β1, which may be triggered by degraded wound ECM components present in chronic wounds [57]. CCNs are ligands of many integrins and the balance between CCN1 and CCN2 appears to affect the outcome of wound healing [55, 57]. In chronic wound healing, CCN1 expression is increased and that of CCN2 is reduced; this excessive amount of CCN1 may lead to premature, α6β1-mediated myofibroblast senescence, resulting in an ECM that is insufficient for reepithelialization. On the other hand, an elevated level of CCN2 is a hallmark of fibrosis and CCN2 may boost myofibroblast functions via TGF-β, leading to a sustained fibrotic response.

## INTEGRINS AND MYOFIBROBLASTS IN ORGAN FIBROSIS

Fibrosis results from an aberrant tissue response to injury characterized by excessive deposition of collagen fibers, ECM remodeling, and tissue scarring. From a mechanistic point of view, a hallmark of fibrosis is the persistent presence of active myofibroblasts that synthesize copious ECM proteins and mediate the remodeling and contraction of the new ECM. A role of integrins in myofibroblast activation, migration, ECM production, and resistance to senescence and apoptosis during tissue fibrosis is much anticipated, but has not been extensively studied until recent years, compared with the extensive research on the role of these proteins in wound healing.

As discussed above, dysregulation of certain integrin isoforms, such as α6β1, may contribute to the failure of myofibroblasts to become senescent and apoptotic by affecting the balance and signaling of CCN1 and CCN2, which leads to the abnormally persistent functioning of myofibroblasts during tissue fibrosis.

β1 integrin is known to be promiscuous, forming heterodimers with many α partners. In these configurations, the α subunit likely imparts ligand specificity to allow each heterodimer to bind a specific ligand, such as collagen or fibronectin. The β1-containing integrins are broadly expressed in many cell types, further increasing the functional diversity of β1 integrins. Notably, fibroblasts from lesioned areas of patients with the chronic fibrotic disorder diffuse scleroderma (diffuse systemic sclerosis, dSSc) show an enhanced ability to adhere to and contract ECM, compared with fibroblasts from nonlesioned areas of dSSc patients and dermal fibroblasts from healthy individuals [58]. Moreover, β1 integrin is overexpressed in lesioned SSc fibroblasts and a neutralizing antibody specific to β1 reverses the excess adhesion to and contraction of ECM by SSc fibroblasts. These findings suggest that β1 is involved in fibrogenesis associated with human fibrosing diseases such as SSc. This notion was confirmed in a mouse model of skin scleroderma with a fibroblast-specific deletion of β1 integrin [28]. Subcutaneous injection of bleomycin, a fibrogenic anticancer drug, induced marked cutaneous thickening, subcutaneous fibrosis, and increased numbers of α-SMA-positive myofibroblasts, whereas the deletion of β1 integrin resulted in a resistance to bleomycin-induced formation of scleroderma. Therefore, β1 integrin is required for fibrogenesis in, at least, this mouse model of SSc.

The αv-containing integrins—i.e., αvβ1, αvβ3, αvβ5, αvβ6, and αvβ8—are important members of the RGD subfamily that recognize RGD-containing

ECM proteins, such as fibronectin and vitronectin, and may contribute to the activation of latent TGF-β. TGF-β is believed to be a major profibrogenic cytokine and a central mediator of fibrosis in multiple organs; but it is secreted as a latent complex that is present at a high concentration in and is cross-linked to the ECM during fibrosis. Much of the regulation of TGF-β signaling is based on the activation of this latent TGF-β in tissues. As will be discussed in more detail in the following section, RGD receptor integrins, such as the αv-containing isoforms, can directly bind the RGD motif present in the amino terminal region of the TGF-β gene product known as the latency-associated peptide (LAP) and thereby activate TGF-β (mainly TGF-β1 and TGF-β3) [59, 60]. Indeed, loss of the function of αvβ6 and αvβ8 in mice by way of gene knockout and antibody inhibition causes phenotypes similar to the developmental effects of knockout of TGF-β1 and TGF-β3 [61]; whereas, activation of TGF-β by αvβ6 plays an important role in models of fibrosis in the lungs, biliary tract, and kidneys [62-65]. In this latter case, αvβ6 expressed by epithelial cells activates TGF-β locally, which is required for the development of fibrosis in these organ systems. This may explain the αvβ6-dependent epithelial influence on myofibroblast differentiation taking place in the subepithelial region beneath the epithelial cells similarly to that observed in wound healing discussed above [56].

On the other hand, myofibroblasts express several αv-containing integrins themselves and all of these integrins can recognize the same RGD motif and, in certain circumstances, activate latent TGF-β. Moreover, mechanical forces generated by the contractile actomyosin cytoskeleton and transmitted by integrins have been identified as a common mechanism for activating TGF-β [66, 67]. Therefore, it has been proposed that the αv integrins expressed in myofibroblasts play a broader role in fibrosis than epithelial cell-expressed integrins, because they activate TGF-β directly at the site of fibrotic foci formation, which, in many cases, are at a considerable distance from epithelial cells. Indeed, by using a conditional-knockout strategy to inactivate the mouse αv gene in myofibroblasts in multiple organs, it was shown that such depletion of αv integrin protected mice from carbon tetrachloride-induced hepatic fibrosis, as well as bleomycin-induced pulmonary fibrosis and unilateral ureteric obstruction (UUB)-induced renal fibrosis [41]. The knockout strategy effectively targeted myofibroblasts and reduced TGF-β activation by the cells significantly. Furthermore, a pharmacological blockade of αv integrins using a small molecule RGD peptidomimetic antagonist that effectively inhibits all αv

integrins *in vitro* attenuated both liver and lung fibrosis in mice. Therefore, it was concluded that these αv-containing integrins of myofibroblasts constitute a core molecular pathway that regulates organ fibrosis via a common mechanism requiring TGF-β activation.

## MOLECULAR ASPECTS OF MYOFIBROBLAST REGULATION BY INTEGRINS

Structural, molecular, and genetic studies have provided insight into the molecular mechanisms by which integrins mediate their diverse functions, many of which are directly responsible for the regulation of myofibroblasts by integrins, in particular those that mediate chemical and mechanical sensing, bidirectional signaling between the cytoplasm and the pericellular environment, TGF-β activation, and mechanical functions, such as adhesion, movement, and contraction. Here we discuss three aspects of the molecular action of integrins in relation to myofibroblast regulation.

*Integrin activation and bidirectional signaling.* Integrins are bidirectional signaling receptors, which is essential to their function in the regulation of myofibroblasts as well as many other types of cells. Moreover, their activity must be tightly controlled in a time-, space-, and context-dependent manner in order for them to function properly [15, 17].

Inactive integrins take a bent conformation in which the head is bent to the legs and the cytoplasmic tails are associated with inactivators (Figure 1B). Binding of intracellular activator proteins at the cytoplasmic tails initiates the inside-out activation of integrins in a stepwise fasion. Talin and focal adhesion kinase (FAK) are activated by signals at the site of integrin clustering and are among the first to bind to the β integrin tail. This is followed by binding to kindlins, which enhance integrin activation, and the IPP complex (integrin-linked kinase, PINCH, and pavin), which recruits focal adhesion proteins, such as paxillin and α-actin, to link to the cytoskeleton. These intracellular binding events extend the integrin domains through the release of the outer membrane clasp and the inner membrane clasp, which effectively straightens the subunits from the bent, inactive conformation resulting in an increase in integrin affinity for extracellular ligands at the head ectodomains [21]. Outside-in signaling is initated by binding of integrins to their extracellular ligands, such as fibronectin and collagen. The βI domain of a β subunit binds a ligand, together with the β-propeller or the αI domain of an α subunit through

MIDAS at the α-β interface in the headpiece, which induces a transition from a "closed" to an "open" conformation of the head commonly referred to as headpiece opening. Finally, a myosin contraction-dependent generation of force stretches talin to allow the binding of vinculin, leading to increased tension between talin and F-actin, which is crucial for adhesion maturation and full activation of integrins. Whether and how these signaling events differ among different cell types and contexts, in particular, if there is a myofibroblast-specific mechanism of integrin activation, remain to be addressed.

*Integrin-dependent cell adhesion and migration.* Cell-matrix interactions are mediated by adhesion receptors, such as integrins, which initiate and control the formation of multi-protein adhesion structures to connect the actin cytoskeleton of a cell with its ECM [68]. Focal adhesions are the best studied cell-matrix interaction structures. Integrins play critical roles in the initiation, maturation, and turnover of focal adhesions by recruiting many binding proteins including FAK, paxillin, talin, vinculin, and α-actin in the lamellipodium protrusions [69]. Some of the protein interactions between integrins and their binding partners inside or outside the cell have been discussed above. There are compositional and structural differences among various adhesion structures with distinct functions. In these scenarios, integrin-dependent adhesion formation and maturation are highly dynamic and have significant effects on cell functions, such as cell spreading, polarization, migration, secretion, and division, in addition to the sensing of the chemical and physical signals and properties of the ECM including ECM rigidity and surface topography. The myosin II-dependent force generation induces a stronger association between integrin and actin and thereby promotes adhesion maturation. Focal adhesions can also evolve into fibrillar adhesions to promote the reorganization of the ECM.

Adhesions provide two functions in cell migration: (a) generation of traction by linking the ECM to actomyosin filaments and (b) organization of the signaling networks that regulate migration and other cellular functions. In integrin-depedent migration, adhesions assemble and disassemble in a very dymanic and polarized fashion, which is essential for optimum cell speed and directional movement [69]. An optimal migration speed is often obtained when intermediate levels of expression of integrins, such as α5β1 and α2β1, and their ligands take place. Notably, adhesions containing different integrin isoforms may differentially affect the phenotypes of cell movement. For instance, fibronectin-α5β1-mediated adhesions are more dynamic than the αvβ3 adhesions that are associated with more persistent migration. This notion

is supported by the correlation between αvβ3 expression and the invasive phenotype of melanomas.

*Cross-talk with growth factor (GF) signaling and TGF-β activation.* Because integrins themselves don't possess enzymatic activity, instead recruiting signaling proteins and enzymes, the signal transduction and function of integrins largely depend on cross-talk with many other signaling pathways, especially those initiated by growth factors (GFs). Interactions between GF receptors (GFRs) and integrin tails produce inside-out signaling that induces the conversion of the integrin from a low affinity to a high affinity receptor state for binding extracellular ligands. Additionally, GFs can alter the spectrum of integrins expressed in the cell. In a reciprocal fashion, integrins feedback on GFR signaling, as many GFRs are present in the integrin-mediated cell adhesions. Moreover, integrins can modulate GFR signaling by interacting with extracellular GFs or the intracellular downstream processes of GFR signaling.

Among the GFs, TGF-β is recognized as a central regulator of the functions of myofibroblasts as well as many other cells in wound healing and fibrosis. Many of of these regulatory activities involve the interaction between TGF-β and integrins. TGF-β directly promotes myofibroblast development by inducing the expression of α-SMA, some ECM proteins, and several cytoskeletal proteins of the myofibroblast contractile apparatus; moreover, TGF-β is induced and is activated in wound healing and many fibrosing diseases in humans and in animal models [70-78].

As discussed above, TGF-β is secreted in a latent form that is confined within LAP and is further associated with the latent TGF-β-binding protein (LTBP) to form a large latent complex within the ECM. Target cells with LAP RGD-recognition integrins—for instance, αvβ6 in keratinocytes and αvβ5 in fibroblasts—attach to the complex and create a traction force through their actin cytoskeleton, leading to a conformational change in the complex to release the active TGF-β locally [79]. Activated TGF-β then binds to cell surface TGF-β receptors to stimulate myofibroblast differentiation, migration, and ECM production, amongst its many other functions. Mice with a knockin mutation of *Tgfb1* in which the RGD binding site is nonfunctional reproduced the phenotypes of *Tgfb1* knockout mice, supporting an essential role of RGD-binding integrins in TGF-β1 activation [80]. Among the integrins, αvβ6 and αvβ8 are perhaps the physiologically direct activators of TGF-β1 and TGF-β3. The mechanisms by which these two integrins activate TGF-β are significantly different. Activation by αvβ6 requires the cytoplasmic tail of β6 and a

functional actin cytoskeleton, which is consistent with the “traction” model of TGF-β activation where matrix contraction instead of a protease activity causes the release of active TGF-β near the cell surface [81]. On the other hand, activation by αvβ8 does not require its β8 tail and is independent of actin cytoskeletal traction. Rather, αvβ8 presents latent TGF-β to a membrane-bound protease, such as MT1-MMP, to activate TGF-β [82]. Whether and how the activation of TGF-β by integrins contribute to the development of disease phenotypes, such as pathologic wound healing, organ fibrosis, and cancer, remain unclear but would represent a rational target for future research and therapeutic development.

## CONCLUSION

There has been increasing interest in understanding myofibroblast regulation by integrins, which is in part spurred by the recognition of the broad range of functions of both myofibroblasts and integrins and, in particular, the remarkable progress in elucidating the structure, function, and signaling of integrins that has been achieved in the broader science covering mammalian development, matrix biology, immunology, and disease development including cancer, fibrosis, and autoimmunity. The manifold capability of integrins—i.e., direct linking between the contractile cytoskeleton structure and the pericellular environment, sensing of the chemical, structural, and physical signals both outside and inside the cell, bidirectional signaling, cross-talking to multiple signaling pathways, synthesizing and remodeling the ECM, and providing traction and contractility for cell adhesion and movement—makes integrins an appropriate fit for many of the functions of myofibroblasts that center around the synthesis, remodelling, and contraction of the ECM, as has been partly demonstrated in models of wound healing and, to a lesser extent, fibrosis through the use of genetically modified mouse strains. Nevertheless, some basic questions remain to be addressed regarding integrin function and mode of action in myofibroblast function and dynamics. In particular, whether integrins regulate myofibroblasts through myofibroblast-specific signaling pathways and, if so, how it contributes to the abberant functions of myofibroblasts in pathologic matrix production and remodeling remain unclear. Research in these directions would provide new targets for therapeutic development against many diseases

in which myofibroblasts play a role, including chronic wound healing, fibrosis, cancer, and autoimmune dysfunction.

## REFERENCES

[1] Gabbiani G, Ryan GB, Majne G, (1971). Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia*. 27. 549-50.

[2] Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA, (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol*. 3. 349-63.

[3] Klingberg F, Hinz B, White ES, (2013). The myofibroblast matrix: implications for tissue repair and fibrosis. *J. Pathol*. 229. 298-309.

[4] Hinz B, (2007). Formation and function of the myofibroblast during tissue repair. *J. Invest. Dermatol*. 127. 526-37.

[5] Bissell MJ, Hines WC, (2011). Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. *Nat. Med*. 17. 320-9.

[6] De Wever O, Demetter P, Mareel M, Bracke M, (2008). Stromal myofibroblasts are drivers of invasive cancer growth. *Int. J. Cancer*. 123. 2229-38.

[7] Hindman B, Ma Q. Myofibroblasts in cancer and fibrosis: two sides of the same coin? In: Martinez A, editor. Myofibroblasts: form, function and role in disease. New York: Nova Science Publishers, Inc.; 2016.

[8] Kapanci Y, Ribaux C, Chaponnier C, Gabbiani G, (1992). Cytoskeletal features of alveolar myofibroblasts and pericytes in normal human and rat lung. *J. Histochem. Cytochem*. 40. 1955-63.

[9] Yokoi Y, Namihisa T, Kuroda H, Komatsu I, Miyazaki A, Watanabe S et al., (1984). Immunocytochemical detection of desmin in fat-storing cells (Ito cells). *Hepatology*. 4. 709-14.

[10] Charbord P, Lerat H, Newton I, Tamayo E, Gown AM, Singer JW et al., (1990). The cytoskeleton of stromal cells from human bone marrow cultures resembles that of cultured smooth muscle cells. *Exp. Hematol*. 18. 276-82.

[11] Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmouliere A, Varga J et al., (2012). Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *Am. J. Pathol*. 180. 1340-55.

[12] Martin P, (1997). Wound healing--aiming for perfect skin regeneration. *Science*. 276. 75-81.

[13] Desmouliere A, Redard M, Darby I, Gabbiani G, (1995). Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am. J. Pathol*. 146. 56-66.

[14] Sarrazy V, Billet F, Micallef L, Coulomb B, Desmouliere A, (2011). Mechanisms of pathological scarring: role of myofibroblasts and current developments. *Wound Repair Regen.* 19 Suppl. 1. s10-5.

[15] Hynes RO, (2002). Integrins: bidirectional, allosteric signaling machines. *Cell*. 110. 673-87.

[16] Luo BH, Carman CV, Springer TA, (2007). Structural basis of integrin regulation and signaling. *Annu. Rev. Immunol*. 25. 619-47.

[17] Bouvard D, Pouwels J, De Franceschi N, Ivaska J, (2013). Integrin inactivators: balancing cellular functions *in vitro* and *in vivo*. *Nat. Rev. Mol. Cell Biol*. 14. 430-42.

[18] Johnson MS, Lu N, Denessiouk K, Heino J, Gullberg D, (2009). Integrins during evolution: evolutionary trees and model organisms. *Biochim. Biophys. Acta*. 1788. 779-89.

[19] Heise T, Dersch P, (2006). Identification of a domain in Yersinia virulence factor YadA that is crucial for extracellular matrix-specific cell adhesion and uptake. *Proc. Natl. Acad. Sci. US*. 103. 3375-80.

[20] Wong KW, Isberg RR, (2005). Emerging views on integrin signaling via Rac1 during invasin-promoted bacterial uptake. *Curr. Opin. Microbiol*. 8. 4-9.

[21] Campbell ID, Humphries MJ, (2011). Integrin structure, activation, and interactions. *Cold Spring Harb. Perspect. Biol*. 3. a004994.

[22] Xiong JP, Stehle T, Diefenbach B, Zhang R, Dunker R, Scott DL et al., (2001). Crystal structure of the extracellular segment of integrin alpha Vbeta3. *Science*. 294. 339-45.

[23] Xie C, Zhu J, Chen X, Mi L, Nishida N, Springer TA, (2009). Structure of an integrin with an αI domain, complement receptor type 4. *EMBO J*. 29. 666-79.

[24] Gardner H, Kreidberg J, Koteliansky V, Jaenisch R, (1996). Deletion of integrin alpha 1 by homologous recombination permits normal murine development but gives rise to a specific deficit in cell adhesion. *Dev. Biol*. 175. 301-13.

[25] Fassler R, Meyer M, (1995). Consequences of lack of beta 1 integrin gene expression in mice. *Genes Dev*. 9. 1896-908.

[26] Stephens LE, Sutherland AE, Klimanskaya IV, Andrieux A, Meneses J, Pedersen RA et al., (1995). Deletion of beta 1 integrins in mice results in inner cell mass failure and peri-implantation lethality. *Genes Dev*. 9. 1883-95.

[27] Liu S, Xu SW, Blumbach K, Eastwood M, Denton CP, Eckes B et al., (2010). Expression of integrin beta1 by fibroblasts is required for tissue repair *in vivo*. *J. Cell Sci*. 123. 3674-82.

[28] Liu S, Kapoor M, Denton CP, Abraham DJ, Leask A, (2009). Loss of beta1 integrin in mouse fibroblasts results in resistance to skin scleroderma in a mouse model. *Arthritis Rheum*. 60. 2817-21.

[29] Chen J, Diacovo TG, Grenache DG, Santoro SA, Zutter MM, (2002). The alpha(2) integrin subunit-deficient mouse: a multifaceted phenotype including defects of branching morphogenesis and hemostasis. *Am. J. Pathol*. 161. 337-44.

[30] Holtkotter O, Nieswandt B, Smyth N, Muller W, Hafner M, Schulte V et al., (2002). Integrin alpha 2-deficient mice develop normally, are fertile, but display partially defective platelet interaction with collagen. *J. Biol. Chem*. 277. 10789-94.

[31] Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC et al., (1996). Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development*. 122. 3537-47.

[32] Mitchell K, Szekeres C, Milano V, Svenson KB, Nilsen-Hamilton M, Kreidberg JA et al., (2009). Alpha3beta1 integrin in epidermis promotes wound angiogenesis and keratinocyte-to-endothelial-cell crosstalk through the induction of MRP3. *J. Cell Sci*. 122. 1778-87.

[33] Yang JT, Rayburn H, Hynes RO, (1995). Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. *Development*. 121. 549-60.

[34] Yang JT, Rayburn H, Hynes RO, (1993). Embryonic mesodermal defects in alpha 5 integrin-deficient mice. *Development*. 119. 1093-105.

[35] Georges-Labouesse E, Messaddeq N, Yehia G, Cadalbert L, Dierich A, Le Meur M, (1996). Absence of integrin alpha 6 leads to epidermolysis bullosa and neonatal death in mice. *Nat. Genet*. 13. 370-3.

[36] Muller U, Wang D, Denda S, Meneses JJ, Pedersen RA, Reichardt LF, (1997). Integrin alpha8beta1 is critically important for epithelial-mesenchymal interactions during kidney morphogenesis. *Cell*. 88. 603-13.

[37] Huang XZ, Wu JF, Ferrando R, Lee JH, Wang YL, Farese RV, Jr. et al., (2000). Fatal bilateral chylothorax in mice lacking the integrin alpha9beta1. *Mol. Cell. Biol.* 20. 5208-15.

[38] Bengtsson T, Aszodi A, Nicolae C, Hunziker EB, Lundgren-Akerlund E, Fassler R, (2005). Loss of alpha10beta1 integrin expression leads to moderate dysfunction of growth plate chondrocytes. *J. Cell Sci*. 118. 929-36.

[39] Popova SN, Barczyk M, Tiger CF, Beertsen W, Zigrino P, Aszodi A et al., (2007). Alpha11 beta1 integrin-dependent regulation of periodontal ligament function in the erupting mouse incisor. *Mol. Cell. Biol*. 27. 4306-16.

[40] Bader BL, Rayburn H, Crowley D, Hynes RO, (1998). Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. *Cell*. 95. 507-19.

[41] Henderson NC, Arnold TD, Katamura Y, Giacomini MM, Rodriguez JD, McCarty JH et al., (2013). Targeting of alphav integrin identifies a core molecular pathway that regulates fibrosis in several organs. *Nat. Med*. 19. 1617-24.

[42] Hodivala-Dilke KM, McHugh KP, Tsakiris DA, Rayburn H, Crowley D, Ullman-Cullere M et al., (1999). Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. *J. Clin. Invest.* 103. 229-38.

[43] Huang X, Griffiths M, Wu J, Farese RV, Jr., Sheppard D, (2000). Normal development, wound healing, and adenovirus susceptibility in beta5-deficient mice. *Mol. Cell. Biol*. 20. 755-9.

[44] Huang XZ, Wu JF, Cass D, Erle DJ, Corry D, Young SG et al., (1996). 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*. 133. 921-8.

[45] Zhu J, Motejlek K, Wang D, Zang K, Schmidt A, Reichardt LF, (2002). beta8 integrins are required for vascular morphogenesis in mouse embryos. *Development*. 129. 2891-903.

[46] Koivisto L, Heino J, Hakkinen L, Larjava H, (2014). Integrins in Wound Healing. *Adv. Wound Care (New Rochelle)*. 3. 762-83.

[47] Kato A, Okamoto O, Ishikawa K, Sumiyoshi H, Matsuo N, Yoshioka H et al., (2011). Dermatopontin interacts with fibronectin, promotes fibronectin fibril formation, and enhances cell adhesion. *J. Biol. Chem*. 286. 14861-9.

[48] Trebaul A, Chan EK, Midwood KS, (2007). Regulation of fibroblast migration by tenascin-C. *Biochem. Soc. Trans*. 35. 695-7.

[49] Reynolds LE, Conti FJ, Lucas M, Grose R, Robinson S, Stone M et al., (2005). Accelerated re-epithelialization in beta3-integrin-deficient- mice is associated with enhanced TGF-beta1 signaling. *Nat. Med*. 11. 167-74.

[50] Nakayama Y, Kon S, Kurotaki D, Morimoto J, Matsui Y, Uede T, (2010). Blockade of interaction of alpha9 integrin with its ligands hinders the formation of granulation in cutaneous wound healing. *Lab. Invest*. 90. 881-94.

[51] Carracedo S, Lu N, Popova SN, Jonsson R, Eckes B, Gullberg D, (2010). The fibroblast integrin alpha11beta1 is induced in a mechanosensitive manner involving activin A and regulates myofibroblast differentiation. *J. Biol. Chem*. 285. 10434-45.

[52] Lygoe KA, Norman JT, Marshall JF, Lewis MP, (2004). AlphaV integrins play an important role in myofibroblast differentiation. *Wound Repair Regen*. 12. 461-70.

[53] Asano Y, Ihn H, Yamane K, Jinnin M, Tamaki K, (2006). Increased expression of integrin alphavbeta5 induces the myofibroblastic differentiation of dermal fibroblasts. *Am. J. Pathol*. 168. 499-510.

[54] Jones C, Ehrlich HP, (2011). Fibroblast expression of alpha-smooth muscle actin, alpha2beta1 integrin and alphavbeta3 integrin: influence of surface rigidity. *Exp. Mol. Pathol*. 91. 394-9.

[55] Jun JI, Lau LF, (2010). Cellular senescence controls fibrosis in wound healing. *Aging (Albany NY)*. 2. 627-31.

[56] Werner S, Krieg T, Smola H, (2007). Keratinocyte-fibroblast interactions in wound healing. *J. Invest. Dermatol*. 127. 998-1008.

[57] Widgerow AD, (2013). Chronic wounds - is cellular ‘reception’ at fault? Examining integrins and intracellular signalling. *Int. Wound J*. 10. 185-92.

[58] Chen Y, Shi-Wen X, van Beek J, Kennedy L, McLeod M, Renzoni EA et al., (2005). Matrix contraction by dermal fibroblasts requires transforming growth factor-beta/activin-linked kinase 5, heparan sulfate-containing proteoglycans, and MEK/ERK: insights into pathological scarring in chronic fibrotic disease. *Am. J. Pathol*. 167. 1699-711.

[59] Gleizes PE, Munger JS, Nunes I, Harpel JG, Mazzieri R, Noguera I et al., (1997). TGF-beta latency: biological significance and mechanisms of activation. *Stem Cells*. 15. 190-7.

[60] Munger JS, Harpel JG, Gleizes PE, Mazzieri R, Nunes I, Rifkin DB, (1997). Latent transforming growth factor-beta: structural features and mechanisms of activation. *Kidney Int*. 51. 1376-82.

[61] Aluwihare P, Mu Z, Zhao Z, Yu D, Weinreb PH, Horan GS et al., (2009). Mice that lack activity of alphavbeta6- and alphavbeta8-integrins reproduce the abnormalities of Tgfb1- and Tgfb3-null mice. *J. Cell Sci*. 122. 227-32.

[62] Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J et al., (1999). The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell*. 96. 319-28.

[63] Wang B, Dolinski BM, Kikuchi N, Leone DR, Peters MG, Weinreb PH et al., (2007). Role of alphavbeta6 integrin in acute biliary fibrosis. *Hepatology*. 46. 1404-12.

[64] Hahm K, Lukashev ME, Luo Y, Yang WJ, Dolinski BM, Weinreb PH et al., (2007). Alphav beta6 integrin regulates renal fibrosis and inflammation in Alport mouse. *Am J Pathol*. 170. 110-25.

[65] Ma LJ, Yang H, Gaspert A, Carlesso G, Barty MM, Davidson JM et al., (2003). Transforming growth factor-beta-dependent and -independent pathways of induction of tubulointerstitial fibrosis in beta6(-/-) mice. *Am. J. Pathol*. 163. 1261-73.

[66] Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T et al., (2011). Latent TGF-beta structure and activation. *Nature*. 474. 343-9.

[67] Wipff PJ, Rifkin DB, Meister JJ, Hinz B, (2007). Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J. Cell Biol*. 179. 1311-23.

[68] Lock JG, Wehrle-Haller B, Stromblad S, (2008). Cell-matrix adhesion complexes: master control machinery of cell migration. *Semin. Cancer Biol*. 18. 65-76.

[69] Huttenlocher A, Horwitz AR, (2011). Integrins in cell migration. *Cold Spring Harb. Perspect. Biol*. 3. a005074.

[70] Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G, (1993). Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J. Cell. Biol*. 122. 103-11.

[71] Werner S, Grose R, (2003). Regulation of wound healing by growth factors and cytokines. *Physiol. Rev*. 83. 835-70.

[72] Malmstrom J, Lindberg H, Lindberg C, Bratt C, Wieslander E, Delander EL, (2004). Transforming growth factor beta 1 specifically induce

proteins involved in the myofibroblast contractilve apparatus. *Mol. Cell. Proteomics*. 3. 466-77.

[73] Dong J, Ma Q, (2015). Advances in mechanisms and signaling pathways of carbon nanotube toxicity. *Nanotoxicology*. 9. 658-76.

[74] Dong J, Porter DW, Batteli LA, Wolfarth MG, Richardson DL, Ma Q, (2015). Pathologic and molecular profiling of rapid-onset fibrosis and inflammation induced by multi-walled carbon nanotubes. *Arch. Toxicol*. 89. 621-33.

[75] Dong J, Yu X, Porter DW, Battelli LA, Kashon ML, Ma Q, (2016). Common and distinct mechanisms of induced pulmonary fibrosis by particulate and soluble chemical fibrogenic agents. *Arch. Toxicol*. 90. 385-402.

[76] Dong J, Ma Q, (2016). *In vivo* activation of a T helper 2-driven innate immune response in lung fibrosis induced by multi-walled carbon nanotubes. *Arch. Toxicol*. DOI 10.1007/s00204-016-1711-1.

[77] He X, Young SH, Schwegler-Berry D, Chisholm WP, Fernback JE, Ma Q, (2011). Multiwalled carbon nanotubes induce a fibrogenic response by stimulating reactive oxygen species production, activating NF-kappaB signaling, and promoting fibroblast-to-myofibroblast transformation. *Chem. Res. Toxicol*. 24. 2237-48.

[78] He X, Wang L, Szklarz G, Bi Y, Ma Q, (2012). Resveratrol inhibits paraquat-induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. *J. Pharmacol. Exp. Ther*. 342. 81-90.

[79] Munger JS, Sheppard D, (2011). Cross talk among TGF-β signaling pathways, integrins, and the extracellular matrix. *Cold Spring Harb. Perspect. Biol*. 3. a00517.

[80] Yang Z, Mu Z, Dabovic B. Jurukovski V, Yu D, Sung J et al., (2007). Absence of integrin-mediated TGFβ1 activation *in vivo* recapitulates the phenotype of TGFβ1-null mice. *J. Cell Biol.* 176. 787-93.

[81] Annes JP, Chen Y, Munger JS, Rifkin DB, (2004). Integrin αVβ6-mediated activation of latent TGF-β requires the latent TGF-β binding protein-1. *J. Cell Biol.* 165. 723-34.

[82] Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H et al., (2002). The integrin αvβ8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-β1. *J. Cell Biol.* 157. 493-507.

## DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.