Cell. Mol. Life Sci. (2016) 73:1145–1157
DOI 10.1007/s00018-015-2110-0

Cellular and Molecular Life Sciences

REVIEW

The myofibroblast, a key cell in normal and pathological tissue repair

Ian A. Darby[1] · Noraina Zakuan[1] · Fabrice Billet[2,3] · Alexis Desmoulière[2,3]

Received: 16 October 2015 / Revised: 30 November 2015 / Accepted: 1 December 2015 / Published online: 17 December 2015

Abstract Myofibroblasts are characterized by their expression of α-smooth muscle actin, their enhanced contractility when compared to normal fibroblasts and their increased synthetic activity of extracellular matrix proteins. Myofibroblasts play an important role in normal tissue repair processes, particularly in the skin where they were first described. During normal tissue repair, they appear transiently and are then lost via apoptosis. However, the chronic presence and continued activity of myofibroblasts characterize many fibrotic pathologies, in the skin and internal organs including the liver, kidney and lung. More recently, it has become clear that myofibroblasts also play a role in many types of cancer as stromal or cancer-associated myofibroblast. The fact that myofibroblasts are now known to be key players in many pathologies makes understanding their functions, origin and the regulation of their differentiation important to enable them to be regulated in normal physiology and targeted in fibrosis, scarring and cancer.

Keywords α-Smooth muscle actin · Contractility · Extracellular matrix · Excessive scarring · Fibrosis · Cancer stroma · Innervation

✉ Ian A. Darby
ian.darby@rmit.edu.au

✉ Alexis Desmoulière
alexis.desmouliere@unilim.fr

[1] School of Medical Sciences, RMIT University, Bundoora, Melbourne, VIC 3083, Australia

[2] Department of Physiology, Faculty of Pharmacy, University of Limoges, 2 rue du Dr. Marcland, 87025 Limoges Cedex, France

[3] EA 6309 Myelin Maintenance and Peripheral Neuropathies, University of Limoges, 87000 Limoges, France

Abbreviations

ECM	Extracellular matrix
SM	Smooth muscle
EMT	Epithelial mesenchymal transition
HSC	Hepatic stellate cell
TGF	Transforming growth factor
CTGF/CCN2	Connective tissue growth factor
PDGF	Platelet-derived growth factor
ROS	Reactive oxygen species
NOX	NADPH oxidase
MMP	Matrix metalloproteinase
LAP	Latency-associated peptide
CGRP	Calcitonin gene-related peptide

Introduction

The myofibroblast is a cell that appears during physiological and pathological states and is responsible for both tissue contraction and the secretion of extracellular matrix (ECM) during wound healing and in numerous pathologies that are characterized by fibrosis. Recruitment and activation of myofibroblasts and the control of their differentiation, proliferation and death is thus of great importance and central to our understanding of the physiology of normal tissue repair [1] and the pathophysiology of the response to injury and subsequent fibrosis in organs such as the skin [2], liver [3], lung [4], kidney [5], heart [6], skeletal muscle [7] and systemic sclerosis [8]. More recently, it has also become apparent that myofibroblasts play an important role as cancer-associated (myo)fibroblasts in the stromal reaction present in several types of tumours where their presence is also linked to poor prognosis [9–11]. Therefore, understanding what regulates their

behaviour will hopefully provide important clues to aid in the discovery of agents with anti-fibrotic and potentially anti-cancer properties.

The first description of myofibroblasts and the coining of the name come from studies performed on wound repair in the skin. The phenomenon of wound contraction has been recognized now for over 100 years [12], though our understanding of the cell responsible for this contraction really only began in the 1950s. Publications from the 1950s speculated on the nature of the driving force behind wound contraction and variously attributed this to either the ECM via shortening of collagen fibres, to cells at the wound margin or to cells within the granulation tissue itself [13, 14, 15]. Later, it became clear that the source of the contractile force was from cells within the granulation tissue and specifically a wound fibroblast with a distinct phenotype compared to that of normal dermal fibroblasts. This cell was then named the myofibroblast in the early 1970s [16].

Early publications used morphological criteria to characterize the myofibroblasts as different from normal, quiescent dermal fibroblasts based on their smooth muscle (SM) like accumulation of microfilament bundles which were assumed to be contractile while later publications sought to characterize cells based on biochemical characteristics as discussed below.

Morphological and biochemical characteristics of myofibroblast phenotype

The earliest descriptions of myofibroblasts identified ultrastructural specializations which showed some similarity to those of SM cells, in particular bundles of cytoplasmic microfilaments [16]. Further ultrastructural and molecular markers that define myofibroblasts were identified later and include cell–cell and cell–matrix adhesions (for review see [17], stress fibres and α-SM actin expression [18] (Fig. 1). In vivo and in vitro, fibroblasts are present that have prominent bundles of microfilaments in their cytoplasm known as stress fibres, but do not possess α-SM-positive microfilament bundles. These fibroblasts can also be shown in vitro to secrete the splice variant form of fibronectin, ED-A fibronectin. Such cells, which have been termed proto-myofibroblasts exert tractional force in connective tissue and may be induced by mechanical stress, but undergo full differentiation into myofibroblasts only when stimulated by TGF-β. Fully differentiated myofibroblasts exert increased force due to contraction [19, 20]. More recently, expression of the cell–cell adhesion protein OB-cadherin has been shown in vivo and in vitro on myofibroblasts [21]. The lack of expression of late differentiation markers of SM cells has also sometimes been used to define myofibroblasts, though this is not without its problems. SM cells express SM myosin heavy chain, smoothelin and h-caldesmon [22–24] and in general myofibroblasts are negative for these markers. The intermediate filament protein desmin, which is normally expressed in muscle cells, has also been used as a negative marker of myofibroblasts, since under normal circumstances myofibroblasts present during wound healing are desmin-negative. However, in some pathological states of scarring, myofibroblasts have been found to be desmin-positive [25]. Other markers that have been reported to be specific for myofibroblasts are less consistent in their staining, for example, the use of the fibroblast marker FSP-1 (S100A4), though it has been used extensively in studies of renal fibrosis [26]. Therefore, it can be difficult in some situations to distinguish myofibroblasts from other mesenchymal cells that possess similar cytoskeletal features, particularly SM cells and pericytes.

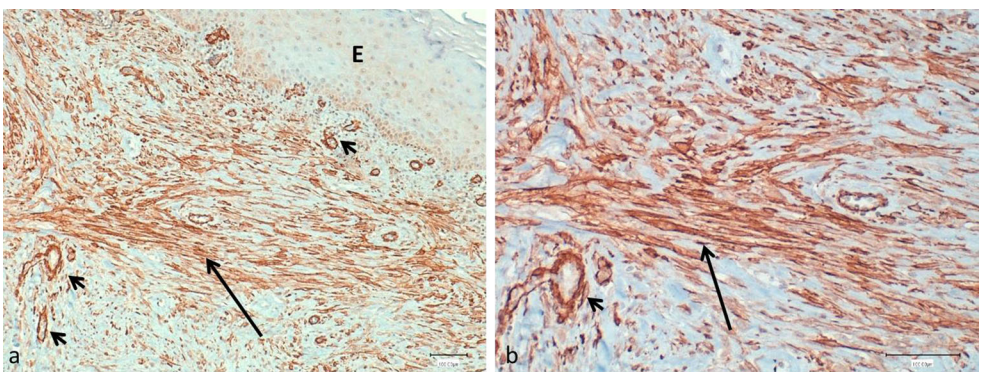

Fig. 1 Human hypertrophic scar tissue stained with α-SM actin antibody. Myofibroblasts persist in nodules in the dermis shown in **a**. The epidermis is indicated by *E*. Small vessels stain positively for α-SM actin (*short arrows*), while myofibroblasts are also positive (*long arrow*). In **b** myofibroblasts are seen as long spindle-shaped cells, often aligned in groups (*long arrow*), while small vessels are also α-SM actin-positive (*short arrow*). *Bar* 100 μm

Cellular origins of myofibroblasts

The origin of myofibroblasts has also been a question that has formed the basis for a considerable amount of research. In most organs that show pathological fibrosis after injury there is a local population of fibroblastic cells that can be drawn on to recruit cells which then differentiate into myofibroblasts. This is particularly so in the skin. In other organs, a variety of cell types may act as sources for recruitment and differentiation of myofibroblasts. In the liver, both portal fibroblasts and hepatic stellate cells can generate myofibroblasts, depending on the site and type of injury that results in myofibroblast recruitment and activation. In the kidney, interstitial fibroblasts are a major source of myofibroblast recruitment. However, other mechanisms have been invoked, specifically epithelial mesenchymal transition (EMT), particularly in the kidney where tubular epithelial cells may, in response to injury and growth factor stimulation, undergo EMT and may be a source of myofibroblasts [27, 28]. In lung and cardiac fibrosis, it has also been suggested that EMT may contribute to the population of myofibroblasts [29]. In studies using mouse models of renal fibrosis, some of the myofibroblasts present in the fibrotic areas were shown to derive from tubular epithelial cells via EMT [30]. However, it remains unclear how important EMT is as a source of myofibroblasts in human pathologies and the proportion derived from local EMT of epithelial cells (or endothelial mesenchymal transition in some cases) may be relatively low. In tumours, the contribution of epithelial cells to the pool of cancer-associated stromal myofibroblasts via EMT may be considerably more significant. In the liver, cell fate studies have suggested that most of the myofibroblasts that appear in the carbon tetrachloride model of liver fibrosis are derived from hepatic stellate cells (HSC) and HSC can be shown to quickly convert from α-SM-negative cells in normal liver to α-SM-positive cells after injury. Though portal fibroblasts would appear to be a likely source of myofibroblasts in fibrotic liver in the case of blockage of the bile duct (cholestasis), in fact cell fate studies again suggest the majority of myofibroblasts in this case again derive from HSC [31]. Lastly, pericytes have been suggested in many cases to be a possible source of myofibroblasts in a number of models of organ fibrosis and cell fate studies in the kidney, for example, have shown pericytes to be an important source of myofibroblasts, and more important than epithelial cell conversion to myofibroblasts via EMT [7, 32].

Regulation of myofibroblast phenotype

The most powerful regulator of myofibroblast phenotype is transforming growth factor (TGF)-β1 which under the right conditions stimulates full conversion of fibroblasts from quiescent α-SM-negative fibroblast or proto-myofibroblast to α-SM-positive myofibroblast [33, 34]. The presence of a splice variant of fibronectin, ED-A fibronectin is necessary for full differentiation into myofibroblasts and the ED-A splice variant which is not expressed by normal endothelial cells or quiescent fibroblasts is also correlated with increased fibrosis [35]. Stimulation of fibroblasts with TGF-β1 induces the expression of α-SM actin and greatly increases collagen synthesis and contractile force. Growth factors that are involved in tissue repair or induced by tissue injury and inflammation have also been shown to have stimulatory effects on myofibroblast proliferation and differentiation, with connective tissue growth factor (CTGF/CCN2) capable of increasing myofibroblast number and matrix deposition, though the presence of TGF-β may be required for full differentiation to α-SM-positive myofibroblasts. Indeed, CTGF/CCN2 may potentiate the effects of TGF-β but be incapable of inducing myofibroblast differentiation on its own [36]. Platelet-derived growth factor (PDGF) is mitogenic for myofibroblasts but does not seem able to induce myofibroblast phenotype on its own either in vivo or in vitro [37, 38]. Blockade of PDGF receptors is anti-fibrotic in the kidney [39, 40] and lung [41] but this relates presumably more to inhibition of proliferation than to blockade of differentiation. PDGF also appears to be necessary for both appearance of pericytes and also the presence of proto-myofibroblasts, giving inhibition of PDGF the potential to reduce myofibroblast appearance via recruitment of pericytes or by blockade of fibroblast to proto-myofibroblast differentiation, thus reducing the population of cells that can undergo full differentiation to myofibroblasts [42, 43]. Both endothelin-1 and angiotensin II have been shown to increase myofibroblast activity and differentiation, probably by induction of TGF-β or by acting in synergy with TGF-β [44, 45]. Similarly, granulocyte macrophage colony stimulating factor has been shown to increase myofibroblasts in vivo, but this is likely due to recruitment and activation of macrophages and again through a concomitant increase in TGF-β availability [38]. The enzyme thrombin can activate myofibroblast phenotype via cleavage of the protease activated receptor. Reactive oxygen species (ROS) have been shown to be a stimulus for myofibroblast activation, with production of ROS through NADPH oxidase (NOX)4, the predominant NOX isoform expressed in myofibroblasts. NOX involvement in myofibroblast activation has been shown in renal, cardiac and lung fibrosis models [46–48]. In vitro, fibroblasts exposed to endoplasmic reticulum stress have recently been shown to increase expression of α-SM actin [49]. It is not clear yet whether this mechanism is active in vivo. Lastly, microRNAs (miRNAs) have also been shown to be involved in myofibroblast induction in

fibrosis and cancer. In particular, miR-21 has been shown to be highly expressed in lung fibrosis and in breast cancer [50, 51]. miR-21 expression appears to correlate with high levels of TGF-β stimulation of myofibroblast phenotype and it has recently been reported that the mechanism for this may be via effects on TGF-β inhibitory pathways, in particular the (inhibitory) Smad7 and phosphatase and tensin homolog [52]. Additionally, other miRNAs may be down-regulated during fibrosis as has been shown for miR-29 in liver fibrosis models, where miR-29 is expressed in hepatic stellate cells but down-regulated during the development of fibrosis [53]. The developing understanding of the role of miRNAs in regulating fibrosis via effects on myofibroblast differentiation and activity makes them a tempting therapeutic target for inhibiting fibrosis.

Effects of mechanical tension

Fibroblasts and myofibroblasts, because of their contractile properties and close relationship with the ECM, can modify their activity depending on the messages received from the mechanical environment [54]. For example, features of myofibroblastic differentiation, such as stress fibres, ED-A fibronectin or α-SM actin expression, appear earlier in granulation tissue that is subjected to an increase in mechanical tension by splinting a full-thickness wound with a plastic frame as compared to normally healing wounds [55]. In aged skin, it is suggested that 'old' fibroblasts have an age-dependent reduction in the capacity for collagen synthesis and therefore simultaneously experience a loss of mechanical stimulation resulting from the decrease in intact collagen fibres and consequent decreased stiffness of the ECM [56, 57].

Fibroblasts cultured on substrates of variable stiffness have also been shown to possess different phenotypes [58]. Cultured fibroblasts do not express stress fibres on soft or compliant surfaces; however, when the stiffness of the substrate increases, a sudden change in cell morphology occurs and stress fibres appear [59]. Other mechanical signals such as shear forces exerted by flow of fluids are able to induce TGF-β1 production and thus differentiation of fibroblasts cultured in collagen gels in the absence of other exterior stimuli such as cytokine treatment [60]. In addition, pre-straining the ECM regulates the bioavailability of TGF-β1 [61]. Thus, the stiff matrix found either in 3D cultures using stiffer (higher concentration) collagen matrix or in vivo in granulation tissue and fibrotic tissues is able to induce full myofibroblast differentiation in concert with TGF-β1 stimulation [62].

The role of mechanical stress in stimulating myofibroblast activity has also been shown in experiments where dermal wounds in mice are mechanically stressed by stretching or splinting the wound; this mechanical load increased myofibroblast activity resulting in increased scar formation, and mimics to some extent the scarring seen in human hypertrophic scars [63]. Conversely, releasing mechanical stress or reducing stiffness has been shown to induce both apoptosis and a reduction in α-SM actin expression and contractility in myofibroblasts [64, 65].

In cancer biology, it is well known that malignant tumours are often stiffer than normal tissue and benign tumours and data suggest that the ECM stiffening correlates with experimental mammary malignancy and likely drives tumour invasion and metastasis. For example, in breast cancer, progression and aggressiveness, collagen linearization and stromal stiffening are all linked and may result from chronic activation of inflammatory pathways and increased TGF-β signalling [66]. Another potentially important deleterious effect of increased numbers of myofibroblasts in tumour stroma is that they have been shown to secrete proteins that increase matrix stiffness, for example, tenascins [67], but also proteins that alter matrix compliance and in addition stimulate cell proliferation and attachment via integrin binding sites, as has been shown for the matricellular protein periostin [68, 69]. Periostin has also been shown to protect cells from apoptosis in a hypoxic environment, as is often the case in tumours. Again, periostin is induced by TGF-β, and may also play a role in establishing a microenvironment that allows establishment of metastatic tumours; the metastatic niche [70]. Thus, myofibroblasts in the stroma of tumours become a target for therapy that might reduce the progression and growth of primary tumours, but also potentially inhibit spread to distant sites and establishment of metastases (Fig. 2).

Both mechanical signalling and stress may modulate myofibroblast differentiation via a number of pathways and mechanisms. Stress may directly activate transcription of the α-SM actin gene, since application of force across integrin binding sites has been shown to up-regulate α-SM actin promoter activity [71].

As mentioned above, mechanical force alone is not generally sufficient to induce myofibroblast differentiation and other factors are needed to act in concert, specifically TGF-β1. Both mechanical signalling and TGF-β1 stimulation increase collagen gene expression by fibroblasts, emphasizing the role that these factors play in stimulating the pro-fibrotic phenotype as is shown by activated myofibroblasts. TGF-β1 also favours the deposition rather than degradation of ECM proteins by up-regulating inhibitors of matrix degradation including tissue inhibitor of metalloproteinases while decreasing the expression of the matrix metalloproteinases (MMP) themselves [72]. Stimulation of myofibroblasts by TGF-β1 itself is also affected by mechanical forces within the damaged or fibrotic tissue. TGF-β1 released from a variety of inflammatory cells and platelets in the microenvironment of damaged or fibrotic

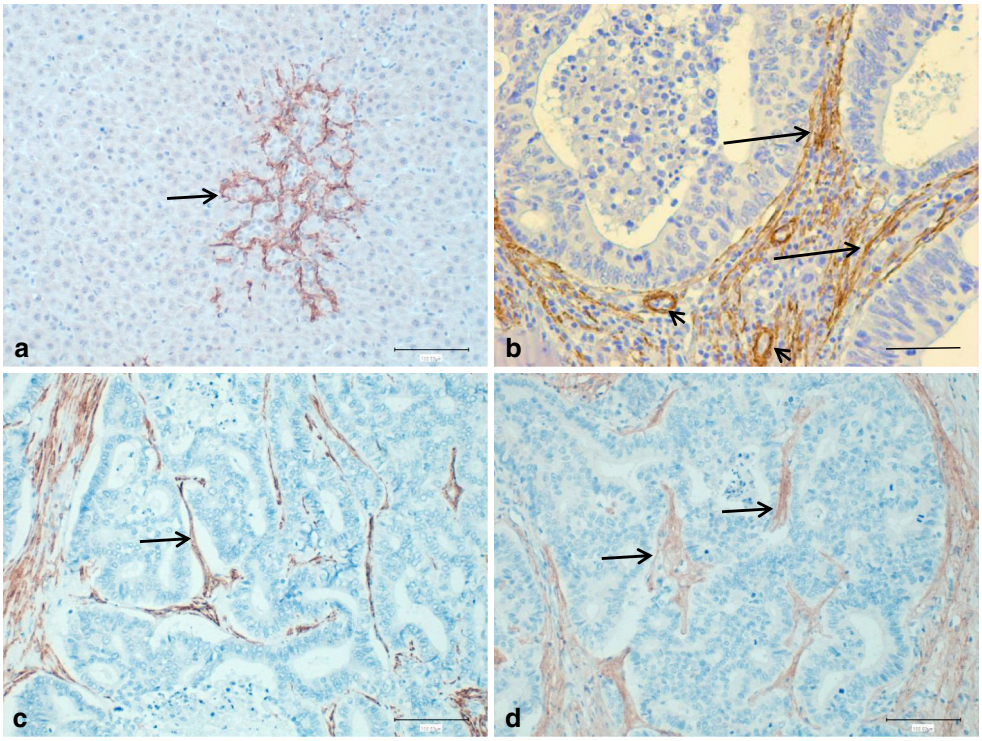

Fig. 2 Myofibroblasts are found in many pathological situations in response to chronic injury or present in the stroma in and around several types of tumour. Myofibroblasts can also be induced by injury such as bile duct ligation or cholestasis in the liver (**a**). Stromal staining of myofibroblasts (indicated by *arrows*) in tumours such as colorectal cancer (**b**) and liver cancer (cholangiocarcinoma) (**c**). In **c** the intimate relationship between tumour cells and myofibroblasts is seen. In many cases, stromal staining of myofibroblasts is a marker of poor prognosis. In addition to their contractile role, myofibroblasts secrete ECM molecules that influence mechanical signalling and cell adhesion. The matricellular protein periostin is secreted by myofibroblasts (positive staining indicated by *arrows*) and has been shown to be important in tumour growth and in establishment of a metastatic niche (**d**). *Bar* 100 μm

tissue is present in a latent form. Indeed, myofibroblasts themselves release latent TGF-β1 complexed with latency-associated peptide (LAP). Together with a binding protein, TGF-β1 is bound to ECM proteins, providing a reservoir of latent TGF-β1 that can be activated as healing and scar formation progress [73, 74]. Latent TGF-β1 can be activated by proteases such as MMP-2 and MMP-9 and by thrombospondin-1 [75, 76]. In addition, myofibroblasts express integrins that can bind to the LAP and mechanical stress applied to the integrins either through mechanical stress on the matrix and/or via myofibroblast contraction can effectively activate TGF-β1 without cleaving the LAP and allow its binding to cell membrane receptors [77]. Thus, both increased mechanical stress and contraction can further increase myofibroblast contractility and matrix protein synthesis. Lastly, it has been shown in vitro that fibroblasts with stress fibres present in their cytoplasm that are α-SM negative can produce tractional forces sufficient to result in contraction of free-floating collagen lattices, but expression of α-SM actin increases contractile force. The evidence that contractile forces can be generated in vivo by fibroblasts that do not express α-SM actin is shown by a recent publication where experimental wounds show some degree of wound contraction in the absence of α-SM actin in α-SM-actin-deficient mice [78].

Effect of innervation and mechanoreceptors

In this section, only skin innervation and its roles during healing, particularly during granulation tissue formation and myofibroblastic differentiation will be discussed. Indeed, the role of innervation in organ repair is poorly known. Sensory as well as autonomic (essentially sympathetic) nerves are present within the skin and influence a variety of physiological and pathophysiological cutaneous functions [79]. In unstimulated nerves, neuromediators are barely detectable within the skin tissues. Upon direct stimulation by physical or chemical means, or during pathological situations such as inflammation or trauma, a significant increase in levels of neuromediators is observed. Thus, mediators derived from sensory or autonomic nerves may play an important regulatory role in the skin under many physiological and pathophysiological conditions, particularly during wound healing.

Cutaneous sensory nerve fibres are endings of dorsal root ganglia (DRG or spinal ganglia) neurons that carry signals from sensory organs toward the appropriate integration centre of the brain via the spinal cord. In the skin, autonomic nerve fibres almost completely derive from sympathetic (cholinergic) neurons. The skin is a highly sensitive organ which is densely innervated with different types of nerve endings that are associated with specific receptors, which discriminate between pain, thermal and tactile sensations [80]. When deep skin damage occurs, cutaneous nerves (sensory and sympathetic nerves) and sensory receptors are destroyed while the sensory and sympathetic neuron cell bodies persist in the ganglia along the spinal cord (respectively, dorsal root ganglia and paravertebral sympathetic ganglia).

Mechanical stimuli are detected via mechanoreceptors associated with sensory corpuscles through Aβ fibres or with Aδ free nerve endings, temperature via the thermoreceptors through Aδ and C fibres, and pain via the nociceptors through Aδ and C fibres (for more details, see review [79]).

Overall, little is known about the role of sensory and autonomic fibres on myofibroblast differentiation and activity. Neurotrophins such as nerve growth factor, neurotrophin-3, brain-derived neurotrophic factor and their receptors are expressed by keratinocytes and melanocytes but also by fibroblasts and myofibroblasts, promoting their proliferation and differentiation [81, 82]. As mentioned above, MMPs secreted by different cells but particularly by (myo)fibroblasts play a major role during the remodelling of the granulation tissue and in scar tissue formation. A recent study has shown that substance P, calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide can modulate MMP-2 and MMP-9 activities; these neuropeptides also affect collagen I and collagen III production during skin wound healing [83]. Thus, neuropeptides such as substance P, CGRP and vasoactive intestinal peptide could have effects on fibroblast to myofibroblast differentiation via effects on both the extracellular matrix composition and mechanical signalling and also via modulation of MMPs and subsequent MMP activation of latent TGF-β1.

Skin lesions and peripheral nerve damage cause resident and infiltrating immune cells, and also the sensory nerve terminals themselves, to release inflammatory mediators including interleukin-1β, tumour necrosis factor-α, bradykinin, substance P, CGRP, nerve growth factor, and prostaglandins, contributing to the "inflammatory soup" [84].

Substance P induces inflammation and mediates angiogenesis, keratinocyte proliferation, and fibrogenesis. Topical application of substance P enhances healing of open excisional wounds in rats [85]. Interestingly, altered substance P levels may contribute to impaired cutaneous healing responses associated with diabetes mellitus or hypertrophic scar formation. In aged rats, application of topical substance P and CGRP were shown to improve wound healing and denervation using capsaicin was shown to inhibit healing [86]. Topical application of exogenous substance P enhances wound closure kinetics in streptozotocin-induced diabetic rats [87] suggesting that diabetic wounds have insufficient substance P levels to promote a neuroinflammatory response necessary for normal wound repair. Conversely, increased nerve numbers and neuropeptide levels with reduced neutral endopeptidase (a membrane-bound metallopeptidase that degrades substance P at the cell membrane) levels in human and porcine hypertrophic scar samples suggest that excessive neuropeptide activity induces exuberant inflammation and ECM deposition with a persistent activation of myofibroblasts in hypertrophic scars [88, 89]. Further to this, Chéret et al. [83] report that the adhesion of human dermal fibroblasts and their differentiation into myofibroblasts are promoted after incubation with vasoactive intestinal peptide, CGRP, and substance P.

It has been shown that wound myofibroblasts may be a target of peripheral nerves, and that delayed wound closure in mature rats is associated with deficiencies in both myofibroblasts and innervation [90]. More recently, Fijiwara et al. demonstrated that direct contact of fibroblasts with neuronal processes is important for differentiation into myofibroblasts and induction of collagen gel contraction, important processes to promote wound healing; the molecular mechanism of fibroblast differentiation by direct contact with neuronal processes was not identified in this study [91].

In addition, oxidopamine (6-OHDA)-induced sympathectomy modifies wound healing with an increase found in wound contraction, a reduction of mast cell migration and a delay of the reepithelialisation; these modifications were associated with a decrease in neurogenic inflammation [92]. It has also been shown that β1- and β2-adrenoceptor blockade impairs cutaneous wound healing [93]. In the liver, in an experimental model of fibrosis using carbon tetrachloride treatment, 6-OHDA-induced denervation significantly reduces matrix deposition and myofibroblast differentiation [94] and acetylcholine promotes both proliferation and collagen gene expression of myofibroblastic hepatic stellate cells [95]. Additionally, cholinergic denervation, obtained via hepatic branch vagotomy or atropine administration, decreases TGF-β1 expression and the proportion of α-SM actin-expressing hepatic stellate cells in carbon tetrachloride-induced liver fibrosis [96].

In clinical research, it has been shown that α-SM actin is detected in hypertrophic scars (see Fig. 1 above) but not in

keloids [97]. Indeed, it seems that α-SM in these both types of excessive scarring is detected [98]. In addition, symptoms of itch and pain, abnormal thermosensory thresholds to warmth as well as cold and heat pain are present in excessive scarring suggesting that these pathological situations are closely associated with small nerve fibres [99].

Moreover, the number of nerve fibres in excessive scarring is significantly higher than in the normal skin samples [100]. Interestingly, in burn patients presenting chronic pain, abnormal cutaneous innervation is observed [101] and very often, in burn patients, hypertrophic scars appear. Finally, in patients with hypertrophic scars, the density of neuropeptide containing nerves was greater in the dermis, compared with normal skin [102].

Inhibition of myofibroblast activity

Since myofibroblasts play a destructive or deleterious role in many fibrotic diseases and in some cancers, there is much interest in blocking myofibroblast activity, reversing or blocking fibroblast phenotypic change or inducing myofibroblast apoptosis. For many years, it was assumed that myofibroblasts could not revert to a normal fibroblast phenotype or at least evidence of this was lacking. It was thus considered most likely that myofibroblasts were perhaps terminally differentiated and if they disappeared, that they did so by apoptosis. It is now becoming apparent, however, that myofibroblasts may in some situations become de-activated and revert to a more normal phenotype [103–105]. Many attempts have been made to target myofibroblasts to develop anti-fibrotic therapies. However, to date no clinically effective compound has been identified, though several are under investigation in the laboratory. Approaches that have been used to inhibit or counteract the action of myofibroblasts are discussed below (Fig. 3).

The presence of myofibroblasts correlates with increased matrix synthesis as mentioned above, and matrix stiffening further stimulates myofibroblast activity by mechanical signalling. It is therefore possible that inhibiting collagen cross-linking and thus increasing ECM compliance may have anti-fibrotic activity by reducing the mechanical signalling to the myofibroblast. One possible means of achieving this is via inhibition of the enzymes involved in collagen cross-linking, specifically lysyl oxidase and lysyl hydroxylases [106].

Several cytokines that partly or fully stimulate myofibroblast differentiation have been described, as discussed above. Additionally, there are some cytokines that have shown inhibition of the myofibroblast phenotype by inhibiting α-SM expression. Interferon-γ has been shown to have beneficial effects on hypertrophic scars and Dupuytren's contracture through inhibition of myofibroblast phenotype [107]. The interferon-γ inducible protein CXCL10 has also been shown to be anti-fibrotic in a mouse model of pulmonary fibrosis and that this action is through inhibition of fibroblast recruitment [108].

Since TGF-β1 is the most important pro-fibrotic growth factor, blockade of TGF-β or TGF-β signalling is another approach that has been used [109]. Inhibition of the TGF-β type I receptor kinase (ALK5) has been shown to reduce fibrosis in several animal models by blocking TGF-β1/Smad signalling and also via an effect on ROS signalling [110]. Similarly, the drug pirfenidone has been shown to reduce fibrosis in several models including the kidney and lung via effects on myofibroblast activity [111]. Pirfenidone has been reported to reduce TGF-β levels, but also inhibits TGF-β-induced phosphorylation of Smad3, p38 and Akt thus reducing TGF-β activation of matrix synthesis and induction of myofibroblast phenotype [112]. The other growth factor that is commonly found to be increased in fibrosis, CTGF/CCN2, can be targeted to reduce fibrosis and CTGF/CCN2-deficient mice show reduced fibrosis in a model of skin fibrosis, for example, [113], making inhibition of CTGF/CCN2 an attractive target for reducing fibrosis.

Another approach has been to inhibit co-factors that are important in the Smad signalling pathway, an example being inhibition of guanosine monophosphate-specific phosphodiesterase 5 (PDE5) which reduces CREB-binding proteins 1 (CBP1) recruitment to Smad transcriptional complexes and thus down-regulates Smad signalling [114, 115]. The pregnancy hormone relaxin has also been reported to inhibit myofibroblast differentiation by inhibiting Smad signalling via Notch1 inhibition of Smad3 and also has a potential anti-fibrotic effect through induction of MMPs [116, 117]. Inhibition of TGF-β-induced serum response factor (SRF) activation, which is required for myofibroblast differentiation, has also been shown to reduce pulmonary fibrosis [118].

Since much of the secreted TGF-β is present in tissue bound to other proteins (such as decorin) and thus in a latent inactive form, one approach to inhibition of TGF-β activity and thus myofibroblast differentiation and activity has been to block activation of latent TGF-β. Blocking integrin binding that is involved in activation of latent TGF-β is one such approach [77, 119, 120]. Inhibition of integrins may also affect attachment of myofibroblasts, mechanical signalling and beta-catenin and Smad signalling thus blockade of several integrin binding sites has been shown to reduce myofibroblast differentiation and activity, for example, blocking of α3β1 integrins [121], α11β1 integrins [122] and αvβ6 integrins [123].

Other pathways that are involved in attachment and mechanical signalling such as focal adhesion kinase have also been targeted and shown to reduce myofibroblast

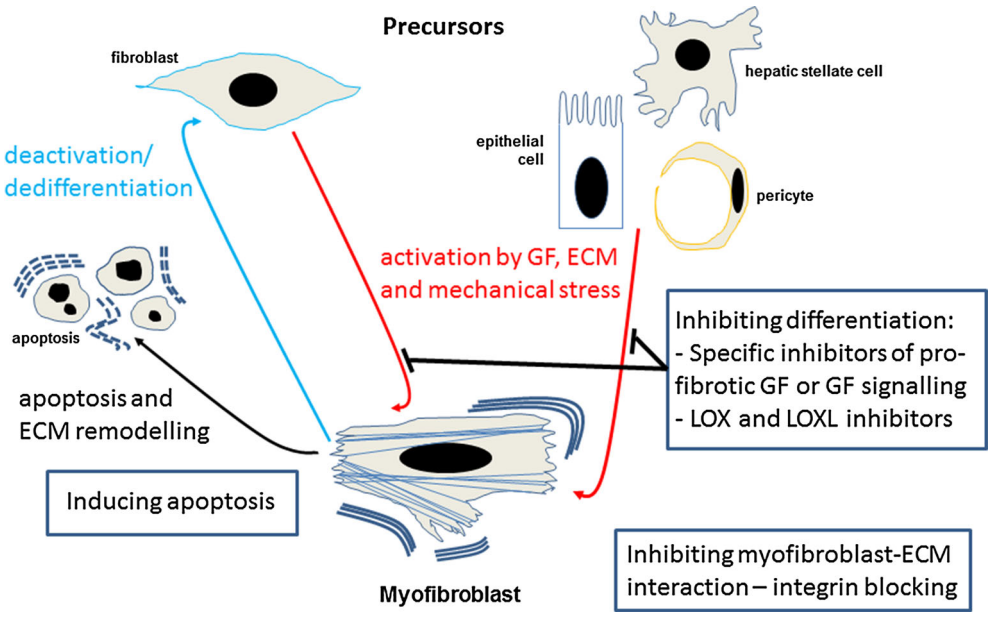

Fig. 3 Myofibroblasts can be derived from various cellular origins, including local fibroblasts, epithelial cells (via EMT), hepatic stellate cells and pericytes. Understanding the regulation of myofibroblast differentiation and survival provides strategies for down-regulating myofibroblast activity and possibly either inducing apoptosis of myofibroblasts or stimulating their dedifferentiation

differentiation in the commonly used bleomycin model of lung fibrosis in mice [124].

Tyrosine kinase inhibition may be able to inhibit fibrosis through down-regulating myofibroblast activation, and the tyrosine kinase inhibitor nintedanib has been shown to reduce the appearance of myofibroblasts in lung fibrosis through reducing tyrosine phosphorylation of the type II TGF-β receptor and thus reducing signalling through Smad3 and p38 mitogen-activated protein kinase [41, 125]. As has been mentioned previously, myofibroblasts in normal wound healing disappear by apoptosis [18, 126] though the mechanism remains unclear. Inducing myofibroblast apoptosis is an attractive prospect for reducing fibrosis and some mediators have been discovered that may accomplish this [127]. Interleukin-1β has been shown to induce apoptosis in myofibroblasts by suppressing inducible nitric oxide synthase (iNOS) expression [128] which may have implications for tissues or organs where macrophage polarization to the M1 pro-inflammatory phenotype means there is high iNOS expression. The other important molecules that are involved in attachment, force transduction and mechanical signalling, are proteins from the actin family and those which interact with the actin cytoskeleton. Molecules that regulate actin assembly could be targeted to modify the cytoskeleton and reduce mechanical signalling and the mammalian Diaphanous-related formins (mDia) is one such molecule [129]. Similarly, inhibition of incorporation of α-SM actin into stress fibres could reduce the tension exerted by fibroblasts on their substratum. This has been achieved though administration of the *N*-terminal actin sequence NH_2-EEED to fibroblasts and produced a reduction in collagen I synthesis. The same N-terminal sequence, administered as a fusion peptide with a cell-penetrating sequence, significantly inhibited the endothelin-induced contractile activity of strips of granulation tissue and delayed the contraction of rat wounds that had been splinted for 10 days [130].

Conclusion and perspectives

The presence and activity of fibroblasts for normal skin homeostasis, and the presence of myofibroblasts for tissue repair is crucial and has evolved to speed normal tissue repair. The importance of fibroblast activity in normal repair has been particularly well documented using in vitro models of dermal substitutes. For example, a living dermal equivalent (containing fibroblasts) applied to skin graft beds was found to reduce pain, to improve hemostasis, and to improve the mechanical and cosmetic properties of the graft; particularly, a normal undulating dermal–epidermal junction reappeared 3–4 months after grafting and elastic fibres were detectable 6–9 months after grafting [131]. Thus, tissue engineering approaches to normal repair require fibroblasts and myofibroblasts to be successful.

However, it is very important to realize that many different populations of (myo)fibroblasts exist and have different properties. For example, interestingly, it has been shown that gingival fibroblasts seem more efficient for remodelling of the connective tissue than dermal fibroblasts [132] and it should be noted that even within organs there is heterogeneity of fibroblasts, for example, fibroblasts from different levels of the skin also show different activities [133].

In addition, it has been shown that, in adults, different mesenchymal stromal/stem cells are able to acquire a (myo)fibroblastic phenotype; including bone marrow-derived mesenchymal stromal/stem cells, but also adipose tissue-derived mesenchymal stromal/stem cells and the cells present in Wharton's jelly around vessels of the umbilical cord. These discoveries offer new perspectives for skin and tissue engineering.

Taking into account the major roles of myofibroblasts in tissue repair, and particularly their contractile properties, the exact mechanisms leading to contraction in a myofibroblast-containing tissue also needs to be clearly identified. By assessing spontaneous intracellular Ca^{2+} oscillations, Follonier et al. have shown that intracellular Ca^{2+} oscillations are coordinated between contacting myofibroblasts via adherens junctions, but randomly between fibroblasts and non-contacting cells [134]. They propose the following model of mechanical coupling for myofibroblasts: individual cell contraction is transmitted via adherens junctions and leads to opening of mechanosensitive ion channels in adjacent cells. The resulting Ca^{2+} influx induces a contraction that can feed back on the first cell and/or stimulate other contacting cells working like a syncytium. This mechanism could improve the remodelling of cell-dense tissue by coordinating the activity of myofibroblasts [135].

Moreover, cancer-associated myofibroblasts, i.e. ECM secreting and contracting stromal cells exhibit α-SM actin-positive stress fibres, play a central role in the detrimental cross-talk between tumour and stroma and almost certainly play a role in tumour metastasis by becoming involved in the metastatic niche for some tumour types. Hence, anti-cancer strategies are now conceivable with the aim being to specifically target myofibroblasts in the tumour stroma [136, 137].

Despite many areas still requiring clarification in myofibroblast biology, it seems clear that myofibroblasts are pivotal cells for the control of ECM deposition and remodelling during normal repair and in pathological situations such as fibrotic scarring and tumour stroma and are definitively an essential target to take into account when developing new therapeutic strategies.

References

1. Darby IA, Hewitson TD (2007) Fibroblast differentiation in wound healing and fibrosis. Int Rev Cytol 257:143–179. doi:10.1016/S0074-7696(07)57004-X
2. Gauglitz GG, Korting HC, Pavicic T, Ruzicka T, Jeschke MG (2011) Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies. Mol Med 17(1–2):113–125. doi:10.2119/molmed.2009.00153
3. Iredale J (2008) Defining therapeutic targets for liver fibrosis: exploiting the biology of inflammation and repair. Pharmacol Res 58(2):129–136. doi:10.1016/j.phrs.2008.06.011
4. Hardie WD, Glasser SW, Hagood JS (2009) Emerging concepts in the pathogenesis of lung fibrosis. Am J Pathol 175(1):3–16. doi:10.2353/ajpath.2009.081170
5. Qi W, Chen X, Holian J, Mreich E, Twigg S, Gilbert RE, Pollock CA (2006) Transforming growth factor-beta1 differentially mediates fibronectin and inflammatory cytokine expression in kidney tubular cells. Am J Physiol Renal Physiol 291(5):F1070–F1077. doi:10.1152/ajprenal.00013.2006
6. Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC (2013) Myofibroblast-mediated mechanisms of pathological remodelling of the heart. Nat Rev Cardiol 10(1):15–26. doi:10.1038/nrcardio.2012.158
7. Dulauroy S, Di Carlo SE, Langa F, Eberl G, Peduto L (2012) Lineage tracing and genetic ablation of ADAM12(+) perivascular cells identify a major source of profibrotic cells during acute tissue injury. Nat Med 18(8):1262–1270. doi:10.1038/nm.2848
8. Castelino FV, Varga J (2014) Emerging cellular and molecular targets in fibrosis: implications for scleroderma pathogenesis and targeted therapy. Curr Opin Rheumatol 26(6):607–614. doi:10.1097/BOR.0000000000000110
9. Cox TR, Erler JT (2011) Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Model Mech 4(2):165–178. doi:10.1242/dmm.004077
10. Pickup MW, Mouw JK, Weaver VM (2014) The extracellular matrix modulates the hallmarks of cancer. EMBO Rep 15(12):1243–1253. doi:10.15252/embr.201439246
11. Tsujino T, Seshimo I, Yamamoto H, Ngan CY, Ezumi K, Takemasa I, Ikeda M, Sekimoto M, Matsuura N, Monden M (2007) Stromal myofibroblasts predict disease recurrence for colorectal cancer. Clin Cancer Res 13(7):2082–2090. doi:10.1158/1078-0432.CCR-06-2191
12. Carrel A, Hartmann A (1916) Cicatrization of wounds: I. The relation between the size of a wound and the rate of its cicatrization. J Exp Med 24(5):429–450
13. Abercrombie M, Flint M, James D (1954) Collagen formation and wound contraction during repair of small excised wounds in the skin of rats. J Embryol Exp Morphol 2:264–274
14. Billingham RE, Russell PS (1956) Studies on wound healing, with special reference to the phenomenon of contracture in experimental wounds in rabbits' skin. Ann Surg 144(6):961–981
15. Watts GT, Grillo HC, Gross J (1958) Studies in wound healing: II. The role of granulation tissue in contraction. Ann Surg 148(2):153–160
16. Gabbiani G, Ryan GB, Majno G (1971) Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia 27(5):549–550
17. Eyden B (2008) The myofibroblast: phenotypic characterization as a prerequisite to understanding its functions in translational medicine. J Cell Mol Med 12(1):22–37. doi:10.1111/j.1582-4934.2007.00213.x

18. Darby I, Skalli O, Gabbiani G (1990) Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest 63(1):21–29
19. 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(5):349–363. doi:10.1038/nrm809
20. Hinz B (2015) Myofibroblasts. Exp Eye Res. doi:10.1016/j.exer.2015.07.009
21. Hinz B, Pittet P, Smith-Clerc J, Chaponnier C, Meister JJ (2004) Myofibroblast development is characterized by specific cell-cell adherens junctions. Mol Biol Cell 15(9):4310–4320. doi:10.1091/mbc.E04-05-0386
22. Benzonana G, Skalli O, Gabbiani G (1988) Correlation between the distribution of smooth muscle or non muscle myosins and alpha-smooth muscle actin in normal and pathological soft tissues. Cell Motil Cytoskelet 11(4):260–274. doi:10.1002/cm.970110405
23. van der Loop FT, Schaart G, Timmer ED, Ramaekers FC, van Eys GJ (1996) Smoothelin, a novel cytoskeletal protein specific for smooth muscle cells. J Cell Biol 134(2):401–411
24. Eyden B (2005) The myofibroblast: a study of normal, reactive and neoplastic tissues, with an emphasis on ultrastructure. J Submicrosc Cytol Pathol 37:109–204
25. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G (1986) A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 103(6 Pt 2):2787–2796
26. Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, Neilson EG (1995) Identification and characterization of a fibroblast marker: FSP1. J Cell Biol 130(2):393–405
27. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG (2002) Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest 110(3):341–350. doi:10.1172/JCI15518
28. Zeisberg M, Kalluri R (2008) Fibroblasts emerge via epithelial-mesenchymal transition in chronic kidney fibrosis. Front Biosci 13:6991–6998
29. Chilosi M, Poletti V, Zamo A, Lestani M, Montagna L, Piccoli P, Pedron S, Bertaso M, Scarpa A, Murer B, Cancellieri A, Maestro R, Semenzato G, Doglioni C (2003) Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. Am J Pathol 162(5):1495–1502
30. Ng YY, Huang TP, Yang WC, Chen ZP, Yang AH, Mu W, Nikolic-Paterson DJ, Atkins RC, Lan HY (1998) Tubular epithelial-myofibroblast transdifferentiation in progressive tubulointerstitial fibrosis in 5/6 nephrectomized rats. Kidney Int 54(3):864–876. doi:10.1046/j.1523-1755.1998.00076.x
31. Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, Pradere JP, Schwabe RF (2013) Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. Nat Commun 4:2823. doi:10.1038/ncomms3823
32. Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, Duffield JS (2010) Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol 176(1):85–97. doi:10.2353/ajpath.2010.090517
33. 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(1):103–111
34. Ronnov-Jessen L, Petersen OW (1993) Induction of alpha-smooth muscle actin by transforming growth factor-beta 1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. Lab Invest 68(6):696–707
35. Shinde AV, Kelsh R, Peters JH, Sekiguchi K, Van De Water L, McKeown-Longo PJ (2015) The alpha4beta1 integrin and the EDA domain of fibronectin regulate a profibrotic phenotype in dermal fibroblasts. Matrix Biol 41:26–35. doi:10.1016/j.matbio.2014.11.004
36. Brigstock DR (2010) Connective tissue growth factor (CCN2, CTGF) and organ fibrosis: lessons from transgenic animals. J Cell Commun Signal 4(1):1–4. doi:10.1007/s12079-009-0071-5
37. Desmouliere A, Rubbia-Brandt L, Grau G, Gabbiani G (1992) Heparin induces alpha-smooth muscle actin expression in cultured fibroblasts and in granulation tissue myofibroblasts. Lab Invest 67(6):716–726
38. Rubbia-Brandt L, Sappino AP, Gabbiani G (1991) Locally applied GM-CSF induces the accumulation of alpha-smooth muscle actin containing myofibroblasts. Virchows Arch B Cell Pathol Incl Mol Pathol 60(2):73–82
39. Chen YT, Chang FC, Wu CF, Chou YH, Hsu HL, Chiang WC, Shen J, Chen YM, Wu KD, Tsai TJ, Duffield JS, Lin SL (2011) Platelet-derived growth factor receptor signaling activates pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis. Kidney Int 80(11):1170–1181. doi:10.1038/ki.2011.208
40. LeBleu VS, Kalluri R (2011) Blockade of PDGF receptor signaling reduces myofibroblast number and attenuates renal fibrosis. Kidney Int 80(11):1119–1121. doi:10.1038/ki.2011.300
41. Wollin L, Maillet I, Quesniaux V, Holweg A, Ryffel B (2014) Antifibrotic and anti-inflammatory activity of the tyrosine kinase inhibitor nintedanib in experimental models of lung fibrosis. J Pharmacol Exp Ther 349(2):209–220. doi:10.1124/jpet.113.208223
42. Bostrom H, Willetts K, Pekny M, Leveen P, Lindahl P, Hedstrand H, Pekna M, Hellstrom M, Gebre-Medhin S, Schalling M, Nilsson M, Kurland S, Tornell J, Heath JK, Betsholtz C (1996) PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. Cell 85(6):863–873
43. Lindahl P, Johansson BR, Leveen P, Betsholtz C (1997) Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 277(5323):242–245
44. Sun G, Stacey MA, Bellini A, Marini M, Mattoli S (1997) Endothelin-1 induces bronchial myofibroblast differentiation. Peptides 18(9):1449–1451
45. Campbell SE, Katwa LC (1997) Angiotensin II stimulated expression of transforming growth factor-beta1 in cardiac fibroblasts and myofibroblasts. J Mol Cell Cardiol 29(7):1947–1958. doi:10.1006/jmcc.1997.0435
46. Cucoranu I, Clempus R, Dikalova A, Phelan PJ, Ariyan S, Dikalov S, Sorescu D (2005) NAD(P)H oxidase 4 mediates transforming growth factor-beta1-induced differentiation of cardiac fibroblasts into myofibroblasts. Circ Res 97(9):900–907. doi:10.1161/01.RES.0000187457.24338.3D
47. Djamali A, Vidyasagar A, Adulla M, Hullett D, Reese S (2009) Nox-2 is a modulator of fibrogenesis in kidney allografts. Am J Transplant 9(1):74–82. doi:10.1111/j.1600-6143.2008.02463.x
48. Hecker L, Vittal R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC, Pennathur S, Martinez FJ, Thannickal VJ (2009) NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. Nat Med 15(9):1077–1081. doi:10.1038/nm.2005
49. Matsuzaki S, Hiratsuka T, Taniguchi M, Shingaki K, Kubo T, Kiya K, Fujiwara T, Kanazawa S, Kanematsu R, Maeda T, Takamura H, Yamada K, Miyoshi K, Hosokawa K, Tohyama M, Katayama T (2015) Physiological ER stress mediates the differentiation of fibroblasts. PLoS One 10(4):e0123578. doi:10.1371/journal.pone.0123578

50. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, Kaminski N, Abraham E (2010) miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J Exp Med 207(8):1589–1597. doi:10.1084/jem.20100035
51. Gong C, Nie Y, Qu S, Liao JY, Cui X, Yao H, Zeng Y, Su F, Song E, Liu Q (2014) miR-21 induces myofibroblast differentiation and promotes the malignant progression of breast phyllodes tumors. Cancer Res 74(16):4341–4352. doi:10.1158/0008-5472.CAN-14-0125
52. McClelland AD, Herman-Edelstein M, Komers R, Jha JC, Winbanks CE, Hagiwara S, Gregorevic P, Kantharidis P, Cooper ME (2015) miR-21 promotes renal fibrosis in diabetic nephropathy by targeting PTEN and SMAD7. Clin Sci Lond. doi:10.1042/CS20150427
53. Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, Janssen J, Koppe C, Knolle P, Castoldi M, Tacke F, Trautwein C, Luedde T (2011) Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology 53(1):209–218. doi:10.1002/hep.23922
54. Hinz B (2010) The myofibroblast: paradigm for a mechanically active cell. J Biomech 43(1):146–155. doi:10.1016/j.jbiomech.2009.09.020
55. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G (2001) Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol 159(3):1009–1020. doi:10.1016/S0002-9440(10)61776-2
56. Fisher GJ, Varani J, Voorhees JJ (2008) Looking older: fibroblast collapse and therapeutic implications. Arch Dermatol 144(5):666–672. doi:10.1001/archderm.144.5.666
57. Vedrenne N, Coulomb B, Danigo A, Bonte F, Desmouliere A (2012) The complex dialogue between (myo)fibroblasts and the extracellular matrix during skin repair processes and ageing. Pathol Biol (Paris) 60(1):20–27. doi:10.1016/j.patbio.2011.10.002
58. Achterberg VF, Buscemi L, Diekmann H, Smith-Clerc J, Schwengler H, Meister JJ, Wenck H, Gallinat S, Hinz B (2014) The nano-scale mechanical properties of the extracellular matrix regulate dermal fibroblast function. J Invest Dermatol 134(7):1862–1872. doi:10.1038/jid.2014.90
59. Yeung T, Georges PC, Flanagan LA, Marg B, Ortiz M, Funaki M, Zahir N, Ming W, Weaver V, Janmey PA (2005) Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. Cell Motil Cytoskelet 60(1):24–34. doi:10.1002/cm.20041
60. Ng CP, Hinz B, Swartz MA (2005) Interstitial fluid flow induces myofibroblast differentiation and collagen alignment in vitro. J Cell Sci 118(Pt 20):4731–4739. doi:10.1242/jcs.02605
61. Klingberg F, Chow ML, Koehler A, Boo S, Buscemi L, Quinn TM, Costell M, Alman BA, Genot E, Hinz B (2014) Prestress in the extracellular matrix sensitizes latent TGF-beta1 for activation. J Cell Biol 207(2):283–297. doi:10.1083/jcb.201402006
62. Goffin JM, Pittet P, Csucs G, Lussi JW, Meister JJ, Hinz B (2006) Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. J Cell Biol 172(2):259–268. doi:10.1083/jcb.200506179
63. Aarabi S, Bhatt KA, Shi Y, Paterno J, Chang EI, Loh SA, Holmes JW, Longaker MT, Yee H, Gurtner GC (2007) Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. FASEB J 21(12):3250–3261. doi:10.1096/fj.07-8218com
64. Grinnell F, Ho CH, Lin YC, Skuta G (1999) Differences in the regulation of fibroblast contraction of floating versus stressed collagen matrices. J Biol Chem 274(2):918–923
65. Wipff PJ, Rifkin DB, Meister JJ, Hinz B (2007) Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. J Cell Biol 179(6):1311–1323. doi:10.1083/jcb.200704042
66. Acerbi I, Cassereau L, Dean I, Shi Q, Au A, Park C, Chen YY, Liphardt J, Hwang ES, Weaver VM (2015) Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. Integr Biol Camb. doi:10.1039/c5ib00040h
67. Orend G, Chiquet-Ehrismann R (2006) Tenascin-C induced signaling in cancer. Cancer Lett 244(2):143–163. doi:10.1016/j.canlet.2006.02.017
68. Oskarsson T, Massague J (2012) Extracellular matrix players in metastatic niches. EMBO J 31(2):254–256. doi:10.1038/emboj.2011.469
69. Darby IA, Vuillier-Devillers K, Pinault E, Sarrazy V, Lepreux S, Balabaud C, Bioulac-Sage P, Desmouliere A (2010) Proteomic analysis of differentially expressed proteins in peripheral cholangiocarcinoma. Cancer Microenviron 4(1):73–91. doi:10.1007/s12307-010-0047-2
70. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J (2012) Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 481(7379):85–89. doi:10.1038/nature10694
71. Wang J, Chen H, Seth A, McCulloch CA (2003) Mechanical force regulation of myofibroblast differentiation in cardiac fibroblasts. Am J Physiol Heart Circ Physiol 285(5):H1871–H1881. doi:10.1152/ajpheart.00387.2003
72. Leask A, Abraham DJ (2004) TGF-beta signaling and the fibrotic response. FASEB J 18(7):816–827. doi:10.1096/fj.03-1273rev
73. Annes JP, Chen Y, Munger JS, Rifkin DB (2004) Integrin alphaVbeta6-mediated activation of latent TGF-beta requires the latent TGF-beta binding protein-1. J Cell Biol 165(5):723–734. doi:10.1083/jcb.200312172
74. Buscemi L, Ramonet D, Klingberg F, Formey A, Smith-Clerc J, Meister JJ, Hinz B (2011) The single-molecule mechanics of the latent TGF-beta1 complex. Curr Biol 21(24):2046–2054. doi:10.1016/j.cub.2011.11.037
75. Yu Q, Stamenkovic I (2000) Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 14(2):163–176
76. Schultz-Cherry S, Murphy-Ullrich JE (1993) Thrombospondin causes activation of latent transforming growth factor-beta secreted by endothelial cells by a novel mechanism. J Cell Biol 122(4):923–932
77. Wipff PJ, Hinz B (2008) Integrins and the activation of latent transforming growth factor beta1—an intimate relationship. Eur J Cell Biol 87(8–9):601–615. doi:10.1016/j.ejcb.2008.01.012
78. Ibrahim MM, Chen L, Bond JE, Medina MA, Ren L, Kokosis G, Selim AM, Levinson H (2015) Myofibroblasts contribute to but are not necessary for wound contraction. Lab Invest. doi:10.1038/labinvest.2015.116
79. Roosterman D, Goerge T, Schneider SW, Bunnett NW, Steinhoff M (2006) Neuronal control of skin function: the skin as a neuroimmunoendocrine organ. Physiol Rev 86(4):1309–1379. doi:10.1152/physrev.00026.2005
80. McGlone F, Reilly D (2010) The cutaneous sensory system. Neurosci Biobehav Rev 34(2):148–159. doi:10.1016/j.neubiorev.2009.08.004
81. Palazzo E, Marconi A, Truzzi F, Dallaglio K, Petrachi T, Humbert P, Schnebert S, Perrier E, Dumas M, Pincelli C (2012) Role of neurotrophins on dermal fibroblast survival and differentiation. J Cell Physiol 227(3):1017–1025. doi:10.1002/jcp.22811

82. Botchkarev VA, Yaar M, Peters EM, Raychaudhuri SP, Botchkareva NV, Marconi A, Raychaudhuri SK, Paus R, Pincelli C (2006) Neurotrophins in skin biology and pathology. J Invest Dermatol 126(8):1719–1727. doi:10.1038/sj.jid.5700270
83. Cheret J, Lebonvallet N, Buhe V, Carre JL, Misery L, Le Gall-Ianotto C (2014) Influence of sensory neuropeptides on human cutaneous wound healing process. J Dermatol Sci 74(3):193–203. doi:10.1016/j.jdermsci.2014.02.001
84. Ellis A, Bennett DL (2013) Neuroinflammation and the generation of neuropathic pain. Br J Anaesth 111(1):26–37. doi:10.1093/bja/aet128
85. Kant V, Gopal A, Kumar D, Bag S, Kurade NP, Kumar A, Tandan SK, Kumar D (2013) Topically applied substance P enhanced healing of open excision wound in rats. Eur J Pharmacol 715(1–3):345–353. doi:10.1016/j.ejphar.2013.04.042
86. Khalil Z, Helme R (1996) Sensory peptides as neuromodulators of wound healing in aged rats. J Gerontol A Biol Sci Med Sci 51(5):B354–B361
87. Kant V, Kumar D, Kumar D, Prasad R, Gopal A, Pathak NN, Kumar P, Tandan SK (2015) Topical application of substance P promotes wound healing in streptozotocin-induced diabetic rats. Cytokine 73(1):144–155. doi:10.1016/j.cyto.2014.12.015
88. Scott JR, Muangman P, Gibran NS (2007) Making sense of hypertrophic scar: a role for nerves. Wound Repair Regen 15(Suppl 1):S27–S31. doi:10.1111/j.1524-475X.2007.00222.x
89. Akaishi S, Ogawa R, Hyakusoku H (2008) Keloid and hypertrophic scar: neurogenic inflammation hypotheses. Med Hypotheses 71(1):32–38. doi:10.1016/j.mehy.2008.01.032
90. Liu M, Warn JD, Fan Q, Smith PG (1999) Relationships between nerves and myofibroblasts during cutaneous wound healing in the developing rat. Cell Tissue Res 297(3):423–433
91. Fujiwara T, Kubo T, Kanazawa S, Shingaki K, Taniguchi M, Matsuzaki S, Gurtner GC, Tohyama M, Hosokawa K (2013) Direct contact of fibroblasts with neuronal processes promotes differentiation to myofibroblasts and induces contraction of collagen matrix in vitro. Wound Repair Regen 21(4):588–594. doi:10.1111/wrr.12059
92. Souza BR, Cardoso JF, Amadeu TP, Desmouliere A, Costa AM (2005) Sympathetic denervation accelerates wound contraction but delays reepithelialization in rats. Wound Repair Regen 13(5):498–505. doi:10.1111/j.1067-1927.2005.00070.x
93. Souza BR, Santos JS, Costa AM (2006) Blockade of beta1- and beta2-adrenoceptors delays wound contraction and re-epithelialization in rats. Clin Exp Pharmacol Physiol 33(5–6):421–430. doi:10.1111/j.1440-1681.2006.04383.x
94. Dubuisson L, Desmouliere A, Decourt B, Evade L, Bedin C, Boussarie L, Barrier L, Vidaud M, Rosenbaum J (2002) Inhibition of rat liver fibrogenesis through noradrenergic antagonism. Hepatology 35(2):325–331. doi:10.1053/jhep.2002.31166
95. Oben JA, Yang S, Lin H, Ono M, Diehl AM (2003) Acetylcholine promotes the proliferation and collagen gene expression of myofibroblastic hepatic stellate cells. Biochem Biophys Res Commun 300(1):172–177
96. Lam HB, Yeh CH, Cheng KC, Hsu CT, Cheng JT (2008) Effect of cholinergic denervation on hepatic fibrosis induced by carbon tetrachloride in rats. Neurosci Lett 438(1):90–95. doi:10.1016/j.neulet.2008.04.048
97. Ehrlich HP, Desmouliere A, Diegelmann RF, Cohen IK, Compton CC, Garner WL, Kapanci Y, Gabbiani G (1994) Morphological and immunochemical differences between keloid and hypertrophic scar. Am J Pathol 145(1):105–113
98. Lee JY, Yang CC, Chao SC, Wong TW (2004) Histopathological differential diagnosis of keloid and hypertrophic scar. Am J Dermatopathol 26(5):379–384
99. Lee SS, Yosipovitch G, Chan YH, Goh CL (2004) Pruritus, pain, and small nerve fiber function in keloids: a controlled study. J Am Acad Dermatol 51(6):1002–1006. doi:10.1016/j.jaad.2004.07.054
100. Hochman B, Nahas FX, Sobral CS, Arias V, Locali RF, Juliano Y, Ferreira LM (2008) Nerve fibres: a possible role in keloid pathogenesis. Br J Dermatol 158(3):651–652. doi:10.1111/j.1365-2133.2007.08401.x
101. Hamed K, Giles N, Anderson J, Phillips JK, Dawson LF, Drummond P, Wallace H, Wood FM, Rea SM, Fear MW (2011) Changes in cutaneous innervation in patients with chronic pain after burns. Burns 37(4):631–637. doi:10.1016/j.burns.2010.11.010
102. Crowe R, Parkhouse N, McGrouther D, Burnstock G (1994) Neuropeptide-containing nerves in painful hypertrophic human scar tissue. Br J Dermatol 130(4):444–452
103. Hecker L, Jagirdar R, Jin T, Thannickal VJ (2011) Reversible differentiation of myofibroblasts by MyoD. Exp Cell Res 317(13):1914–1921. doi:10.1016/j.yexcr.2011.03.016
104. Kisseleva T, Cong M, Paik Y, Scholten D, Jiang C, Benner C, Iwaisako K, Moore-Morris T, Scott B, Tsukamoto H, Evans SM, Dillmann W, Glass CK, Brenner DA (2012) Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc Natl Acad Sci USA 109(24):9448–9453. doi:10.1073/pnas.1201840109
105. Talele NP, Fradette J, Davies JE, Kapus A, Hinz B (2015) Expression of alpha-smooth muscle actin determines the fate of mesenchymal stromal cells. Stem Cell Rep 4(6):1016–1030. doi:10.1016/j.stemcr.2015.05.004
106. van der Slot AJ, Zuurmond AM, van den Bogaerdt AJ, Ulrich MM, Middelkoop E, Boers W, Karel Ronday H, DeGroot J, Huizinga TW, Bank RA (2004) Increased formation of pyridinoline cross-links due to higher telopeptide lysyl hydroxylase levels is a general fibrotic phenomenon. Matrix Biol 23(4):251–257. doi:10.1016/j.matbio.2004.06.001
107. Pittet B, Rubbia-Brandt L, Desmouliere A, Sappino AP, Roggero P, Guerret S, Grimaud JA, Lacher R, Montandon D, Gabbiani G (1994) Effect of gamma-interferon on the clinical and biologic evolution of hypertrophic scars and Dupuytren's disease: an open pilot study. Plast Reconstr Surg 93(6):1224–1235
108. Jiang D, Liang J, Campanella GS, Guo R, Yu S, Xie T, Liu N, Jung Y, Homer R, Meltzer EB, Li Y, Tager AM, Goetinck PF, Luster AD, Noble PW (2010) Inhibition of pulmonary fibrosis in mice by CXCL10 requires glycosaminoglycan binding and syndecan-4. J Clin Invest 120(6):2049–2057. doi:10.1172/JCI38644
109. Varga J, Pasche B (2009) Transforming growth factor beta as a therapeutic target in systemic sclerosis. Nat Rev Rheumatol 5(4):200–206. doi:10.1038/nrrheum.2009.26
110. Park SA, Kim MJ, Park SY, Kim JS, Lee SJ, Woo HA, Kim DK, Nam JS, Sheen YY (2015) EW-7197 inhibits hepatic, renal, and pulmonary fibrosis by blocking TGF-beta/Smad and ROS signaling. Cell Mol Life Sci 72(10):2023–2039. doi:10.1007/s00018-014-1798-6
111. Shimizu T, Kuroda T, Hata S, Fukagawa M, Margolin SB, Kurokawa K (1998) Pirfenidone improves renal function and fibrosis in the post-obstructed kidney. Kidney Int 54(1):99–109. doi:10.1046/j.1523-1755.1998.00XXX.x
112. Conte E, Gili E, Fagone E, Fruciano M, Iemmolo M, Vancheri C (2014) Effect of pirfenidone on proliferation, TGF-beta-induced myofibroblast differentiation and fibrogenic activity of primary human lung fibroblasts. Eur J Pharm Sci 58:13–19. doi:10.1016/j.ejps.2014.02.014
113. Liu S, Parapuram SK, Leask A (2013) Fibrosis caused by loss of PTEN expression in mouse fibroblasts is crucially dependent on CCN2. Arthr Rheum 65(11):2940–2944. doi:10.1002/art.38121
114. Gong W, Yan M, Chen J, Chaugai S, Chen C, Wang D (2014) Chronic inhibition of cyclic guanosine monophosphate-specific

phosphodiesterase 5 prevented cardiac fibrosis through inhibition of transforming growth factor beta-induced Smad signaling. Front Med 8(4):445–455. doi:10.1007/s11684-014-0378-3
115. Gonzalez-Cadavid NF, Rajfer J (2010) Treatment of Peyronie's disease with PDE5 inhibitors: an antifibrotic strategy. Nat Rev Urol 7(4):215–221. doi:10.1038/nrurol.2010.24
116. Sassoli C, Chellini F, Pini A, Tani A, Nistri S, Nosi D, Zecchi-Orlandini S, Bani D, Formigli L (2013) Relaxin prevents cardiac fibroblast-myofibroblast transition via notch-1-mediated inhibition of TGF-beta/Smad3 signaling. PLoS One 8(5):e63896. doi:10.1371/journal.pone.0063896
117. Huang X, Gai Y, Yang N, Lu B, Samuel CS, Thannickal VJ, Zhou Y (2011) Relaxin regulates myofibroblast contractility and protects against lung fibrosis. Am J Pathol 179(6):2751–2765. doi:10.1016/j.ajpath.2011.08.018
118. Sandbo N, Lau A, Kach J, Ngam C, Yau D, Dulin NO (2011) Delayed stress fiber formation mediates pulmonary myofibroblast differentiation in response to TGF-beta. Am J Physiol Lung Cell Mol Physiol 301(5):L656–L666. doi:10.1152/ajplung.00166.2011
119. 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(2):499–510
120. Zhou Y, Hagood JS, Lu B, Merryman WD, Murphy-Ullrich JE (2010) Thy-1-integrin alphav beta5 interactions inhibit lung fibroblast contraction-induced latent transforming growth factor-beta1 activation and myofibroblast differentiation. J Biol Chem 285(29):22382–22393. doi:10.1074/jbc.M110.126227
121. Kim KK, Wei Y, Szekeres C, Kugler MC, Wolters PJ, Hill ML, Frank JA, Brumwell AN, Wheeler SE, Kreidberg JA, Chapman HA (2009) Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. J Clin Invest 119(1):213–224. doi:10.1172/JCI36940
122. 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(14):10434–10445. doi:10.1074/jbc.M109.078766
123. Horan GS, Wood S, Ona V, Li DJ, Lukashev ME, Weinreb PH, Simon KJ, Hahm K, Allaire NE, Rinaldi NJ, Goyal J, Feghali-Bostwick CA, Matteson EL, O'Hara C, Lafyatis R, Davis GS, Huang X, Sheppard D, Violette SM (2008) Partial inhibition of integrin alpha(v)beta6 prevents pulmonary fibrosis without exacerbating inflammation. Am J Respir Crit Care Med 177(1):56–65. doi:10.1164/rccm.200706-805OC
124. Lagares D, Busnadiego O, Garcia-Fernandez RA, Kapoor M, Liu S, Carter DE, Abraham D, Shi-Wen X, Carreira P, Fontaine BA, Shea BS, Tager AM, Leask A, Lamas S, Rodriguez-Pascual F (2012) Inhibition of focal adhesion kinase prevents experimental lung fibrosis and myofibroblast formation. Arthr Rheum 64(5):1653–1664. doi:10.1002/art.33482
125. Rangarajan S, Kurundkar A, Kurundkar D, Bernard K, Sanders YY, Ding Q, Antony VB, Zhang J, Zmijewski J, Thannickal VJ (2015) Novel mechanisms for the anti-fibrotic action of nintedanib. Am J Respir Cell Mol Biol. doi:10.1165/rcmb.2014-0445OC
126. 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(1):56–66
127. de Andrade JA, Thannickal VJ (2009) Innovative approaches to the therapy of fibrosis. Curr Opin Rheumatol 21(6):649–655. doi:10.1097/BOR.0b013e328330da9b
128. Zhang HY, Phan SH (1999) Inhibition of myofibroblast apoptosis by transforming growth factor beta(1). Am J Respir Cell Mol Biol 21(6):658–665. doi:10.1165/ajrcmb.21.6.3720
129. Chan MW, Chaudary F, Lee W, Copeland JW, McCulloch CA (2010) Force-induced myofibroblast differentiation through collagen receptors is dependent on mammalian diaphanous (mDia). J Biol Chem 285(12):9273–9281. doi:10.1074/jbc.M109.075218
130. Hinz B, Gabbiani G, Chaponnier C (2002) The NH2-terminal peptide of alpha-smooth muscle actin inhibits force generation by the myofibroblast in vitro and in vivo. J Cell Biol 157(4):657–663. doi:10.1083/jcb.200201049
131. Coulomb B, Friteau L, Baruch J, Guilbaud J, Chretien-Marquet B, Glicenstein J, Lebreton-Decoster C, Bell E, Dubertret L (1998) Advantage of the presence of living dermal fibroblasts within in vitro reconstructed skin for grafting in humans. Plast Reconstr Surg 101(7):1891–1903
132. Chaussain Miller C, Septier D, Bonnefoix M, Lecolle S, Lebreton-Decoster C, Coulomb B, Pellat B, Godeau G (2002) Human dermal and gingival fibroblasts in a three-dimensional culture: a comparative study on matrix remodeling. Clin Oral Investig 6(1):39–50
133. Modarressi A, Pietramaggiori G, Godbout C, Vigato E, Pittet B, Hinz B (2010) Hypoxia impairs skin myofibroblast differentiation and function. J Invest Dermatol 130(12):2818–2827. doi:10.1038/jid.2010.224
134. Follonier L, Schaub S, Meister JJ, Hinz B (2008) Myofibroblast communication is controlled by intercellular mechanical coupling. J Cell Sci 121(Pt 20):3305–3316. doi:10.1242/jcs.024521
135. Follonier Castella L, Gabbiani G, McCulloch CA, Hinz B (2010) Regulation of myofibroblast activities: calcium pulls some strings behind the scene. Exp Cell Res 316(15):2390–2401. doi:10.1016/j.yexcr.2010.04.033
136. Elkhattouti A, Hassan M, Gomez CR (2015) Stromal fibroblast in age-related cancer: role in tumorigenesis and potential as novel therapeutic target. Front Oncol 5:158. doi:10.3389/fonc.2015.00158
137. Zhou L, Yang K, Andl T, Wickett RR, Zhang Y (2015) Perspective of targeting cancer-associated fibroblasts in melanoma. J Cancer 6(8):717–726. doi:10.7150/jca.10865