

## Review

## Evolution and clinical implications of the myofibroblast concept

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**1. Development of the myofibroblast concept**

The phenomena of wound contraction and scar retraction are known since old ages (for review see [1]). In the first part of our century, the work of Carrel and Lecomte du Noüy has contributed to the notion that the forces producing wound contraction are generated within the granulation tissue itself [2]. These forces were generally considered to depend on extracellular matrix rearrangements; however, Abercrombie and co-workers reported, in the fifties, that fibroblasts exert tractional forces *in vitro* [3]. Similarly, Hoffmann-Beerling showed that addition of ATP to permeabilized fibroblasts in culture produces a contraction of their cytoplasm [4]. In this context, and in the context of emerging work on cytoskeleton morphology and function [5], the ultrastructural observation made in our laboratory in 1971 that during granulation tissue evolution fibroblasts acquire smooth muscle (SM) cell features, such as the presence of cytoplasmic microfilament bundles [6], lead to the proposition that these cells are the source of the force producing wound contraction, and probably connective tissue retraction during fibrotic phenomena. Shortly thereafter, it was shown that strips of granulation tissue isolated and placed in a pharmacological bath would contract and relax under the influence of substances which are known to be capable of contracting and relaxing SM cells [7,8]. It is noteworthy (particularly because this observation has never been developed) that granulation tissues from different locations respond differently to the same agonist or antagonist stimulus, suggesting that the capacity to react with contraction to a given stimulus by fibroblastic cells depends on their location [8]. The name myofibroblast was suggested for this modified and possibly contractile fibroblast [7].

During the next few years, several laboratories reported by means of ultrastructural techniques the presence of myofibroblasts in several lesions characterized by fibrosis and retraction such as fibromatoses [9], liver cirrhosis [10], pulmonary [11] and renal [12] fibrosis. The presence of myofibroblasts was also shown in normal tissues in locations where a certain degree of tension was needed to

function, such as the theca externa of the ovary [13], the alveolar septum [14] and the intestinal pericriptal cells [15]. Hence, the concept was developed that in both normal and pathological situations myofibroblasts develop in response to traction stimuli and can exert a force producing tissue resistance and, particularly in pathological cases, tissue deformation (for review see [16]).

Because of the emerging concept that non-muscle cells contain contractile proteins, which up to that time had been considered typical of muscle cells, and because of the description of other cytoskeletal structures, such as microtubules and intermediate filaments, a considerable effort was made by our as well as some other laboratories in the characterization of contractile and cytoskeletal elements present in myofibroblasts (for review see [17]). It was shown that myofibroblasts contain higher amounts of polymerized actin, as compared to normal fibroblasts *in vivo* [18]. It was also shown that normal tissues containing myofibroblasts, such as the lung alveolus, would produce contraction upon the stimulus of agents contracting SM cells [19]. A significant advance was the production of an antibody against  $\alpha$ -SM actin [20], one of the six actin isoforms present in mammalian tissues and considered to be typical for vascular SM cells [21]. The use of this antibody showed that  $\alpha$ -SM actin is expressed temporarily in granulation tissue of an experimental wound and disappears when the scar develops [22]. Thus, an actin isoform typical of SM cells is expressed during wound healing in granulation tissue fibroblasts; this expression coincides with the phenomenon of wound contraction. Studies by several laboratories demonstrated the presence of  $\alpha$ -SM actin in myofibroblastic cells present in fibrotic tissues of different organs such as the liver [23,24], the lung [25], the kidney [26], the heart [27,28] and the breast [29–31]; moreover,  $\alpha$ -SM actin was suggested as the most significant marker of myofibroblastic cells, at least in pathological settings [17]. The presence of myofibroblasts in myocardial granulation tissue following an infarct has been shown by means of ultrastructure [32] to persist for a long time. The finding was confirmed by immunohistochemistry [33]. It is noteworthy that in old myocardial scars,

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fibroblastic cells still express  $\alpha$ -SM actin, probably because of the continuous tension present in these areas [27]. In myocardial, as well as in cutaneous granulation tissue myofibroblasts are spatially highly organized. Recently, it has been shown that the myocardial myofibroblasts express a homologue of drosophila tissue polarity gene, *frizzled*, when they migrate into granulation tissue; this may represent the signal for alignment [28]. Another phenomenon in which the modulation between fibroblast and myofibroblast could play an important role is the fibrosis development during heart failure. Angiotensin II has been suggested to be important for fibrosis formation in this situation [34].

Further work demonstrated that myofibroblasts, according to the pathological situation, may express other markers of SM cells, such as SM myosin heavy chains and desmin, the intermediate filament protein characteristic of muscle cells [16,17]. Other muscular proteins described in myofibroblasts were caldesmon, SM22 and tropomyosin [31].  $\alpha$ -SM actin is the SM cell marker generally present in myofibroblasts of normally healing granulation tissue; other markers appear in more permanent fibrotic situations such as liver cirrhosis and become notably expressed in the stroma reaction to epithelial tumors, e.g. breast cancer [30,31,35,36]. Thus, it is possible to conclude that during the evolution of fibrosis, fibroblasts acquire SM cell features; these appear temporarily in normal wound healing and more permanently in irreversible fibrotic situations. The presence of myofibroblasts was shown to coincide with collagen type III expression in Dupuytren's nodules [37]; this collagen isoform is known to be present in remodeling connective tissue. In addition to these early biochemical observations, more precise *in situ* hybridization studies showed that myofibroblasts, characterized by the expression of  $\alpha$ -SM actin, were the cells responsible for collagen type I mRNA production in pulmonary fibrosis [38].

Studies aimed at the elucidation of the mechanisms regulating the transition between granulation tissue and scar formation demonstrated that apoptosis is the mechanism through which myofibroblasts disappear during this transition [39]. This observation clarified an important aspect of wound healing and suggested that hypertrophic scar formation may be the consequence of a lack of myofibroblast apoptosis at the appropriate time of healing.

The role of myofibroblasts in wound contraction was confirmed by work showing that during fetal wound healing, when regeneration phenomena rather than scarring predominate, there is no appearance of myofibroblasts [33].

## 2. Agents influencing myofibroblast evolution

Another important aspect of myofibroblast research has been the work explaining the factors which influence the development and the disappearance of these cells. Essen-

tially, this work has concentrated on cytokines and growth factors. The observation that  $\gamma$ -interferon decreases the expression of  $\alpha$ -SM actin in SM cells [40] suggested us to verify this possibility in granulation tissue and fibrotic tissues. Indeed,  $\gamma$ -interferon decreases  $\alpha$ -SM actin expression in cultured fibroblasts and *in vivo* as well [41]. The most extensive studies on this subject have been done using the model of liver fibrosis [23,42]. Moreover, pilot clinical studies have suggested that  $\gamma$ -interferon application is efficient in reducing hypertrophic scars and fibromatosis lesions [43].

By testing several cytokines in an experimental model consisting of their topical application to the rat subcutaneous tissue through an osmotic mini pump, it was shown that TNF, IL-1 and PDGF do not stimulate myofibroblast formation, even when they elicit a granulation tissue response, whereas TGF- $\beta$ 1 and - $\beta$ 2, and more surprisingly GM-CSF, regularly stimulate the appearance of  $\alpha$ -SM actin rich myofibroblasts [44,45]. TGF- $\beta$ 1 and - $\beta$ 2 appear to exert a very powerful  $\alpha$ -SM actin synthesis, stimulating activity in cultured fibroblasts [46,47]. If one considers that TGF- $\beta$  is known since a long time to exert a profibrotic activity and specifically to stimulate collagen type I synthesis [48], one can conclude that this cytokine is probably the most critical for the development of scarring and fibrosis. It is noteworthy that TGF- $\beta$  can induce  $\alpha$ -SM actin expression in cultured fibroblastic cells in the absence of serum, that is in the absence of cell replication which indicates a *de novo* synthesis of this protein [46]. The action of GM-CSF appears to be indirect in that, when GM-CSF is applied to fibroblastic cells in culture, no  $\alpha$ -SM actin synthesis is stimulated whereas  $\alpha$ -SM actin rich myofibroblasts are seen after local administration of GM-CSF in the subcutaneous tissue. The same is true when GM-CSF is applied either directly, or through a viral vector to the lung alveoli [49]. Here the administration of GM-CSF is followed by TGF- $\beta$ 1 expression and then by  $\alpha$ -SM actin and collagen expression [49]. Some preliminary research indicates that IL-10 could also play a role in the stimulation of myofibroblast development in the kidney [50]. Taken together, these results suggest that local GM-CSF application could, either directly or indirectly through the accumulation of stimulated macrophages, elicit an important expression of TGF- $\beta$ 1 which in turn would induce  $\alpha$ -SM actin and collagen expression, thus resulting in fibrotic changes or scarring.

A more recent observation in our laboratory has shown that the action of TGF- $\beta$ 1 is mediated through the ED-A portion of cellular fibronectin [51]. This conclusion is based on the observation that antibodies specific for the ED-A sequence inhibit the action of TGF- $\beta$  in inducing both  $\alpha$ -SM actin and collagen mRNA and protein expression. Thus, myofibroblast development probably depends on both cytokine stimulation and extracellular matrix organization. It is noteworthy that TGF- $\beta$ 1 elicits the synthesis of ED-A fibronectin *in vitro* [52].

### 3. Mechanisms of myofibroblast activity

Observations by several laboratories have indicated that fibroblastic cells *in vitro* exert mainly isometric traction forces rather than an isotonic contractile activity [53]. It is probable that a similar mechanism takes place *in vivo* during granulation tissue contraction. It has been postulated that cytoskeletal elements interconnecting different cells are organized as a tensegrity structure in which cytoskeletal components such as microtubules or intermediate filaments exert a resistance to the tension produced by contractile elements such as actin and myosin [54]. In this context, an interesting aspect of myofibroblast research has concerned the role of  $\alpha$ -SM actin in the generation of myofibroblast contractile forces. In addition to being a marker of myofibroblast differentiation,  $\alpha$ -SM actin could be functionally important for myofibroblast contraction if one takes into account the fact that  $\alpha$ -SM actin is expressed throughout the phylogenetic scale in cells whose main function is contraction, such as vascular SM cells or myoepithelial cells. Actin isoforms are very conserved and exhibit relatively small differences in amino acid sequence, which probably correspond to functional differences. We have recently characterized the sequence corresponding to the epitope of the monoclonal antibody against  $\alpha$ -SM actin which is Ac-EEED and shown that this sequence is important for  $\alpha$ -SM actin polymerization [55]. Indeed the incubation of  $\alpha$ -SM actin but not of other actin isoforms with the  $\alpha$ -SM actin specific antibody or with its Fab fragment accelerates  $\alpha$ -SM actin polymerization. Conversely, the microinjection of the epitopic peptide into fibroblastic or SM cells inhibits selectively  $\alpha$ -SM actin polymerization. This observation leads one to conceive the possibility of eliminating selectively  $\alpha$ -SM actin from stress fibers of a given cell and this offers a tool for testing the role of this protein in fibroblastic cell shape maintenance and contractile activity. Recent work has shown that inhibition of  $\alpha$ -SM actin expression in SM cells and/or fibroblasts increases their motility and likely decreases their contractile activity [56]. Hence, it is possible that  $\alpha$ -SM actin plays an important role for the development of myofibroblastic force; if this is verified by *in vivo* experiments, one could envisage the possibility of influencing wound contraction by treating wounds or fibrotic tissues with the  $\alpha$ -SM actin N-terminal peptide.

In conclusion, it appears that during the last 25 years the concept that cell activity is the main source of force generation within granulation tissue has gained general acceptance. How force is produced remains to be determined, but it is increasingly accepted that the presence of  $\alpha$ -SM actin is instrumental for this action. Many of the factors influencing synthesis and disappearance of  $\alpha$ -SM actin are presently known and it is now possible to envisage the study of its role in myofibroblast activity. We are confident that during the next years more data will become available explaining the biological activity of the myofi-

broblast and possibly allowing the modulation of its activity in order to accelerate and reduce wound contraction and fibrosis formation.

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