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LETTURA

## The Evolution of the Myofibroblast Concept: a Key Cell for Wound Healing and fibrotic diseases

### *I processi di riparazione e fibrosi*

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Wound healing and fibrocontractive diseases are characterized by the presence of a cell called myofibroblast that is responsible for pathological tissue remodeling. TGF-beta is the main stimulus for the fibroblast/myofibroblast modulation. Alpha-smooth muscle (SM) actin, the actin isoform typical of vascular smooth muscle cells, is the main marker of the myofibroblastic differentiation and in addition is responsible for the high retractile activity of this cell. The N-terminal sequence of alpha-SM actin inhibits myofibroblast contraction in vitro and in vivo and could represent a therapeutic tool in fibrotic diseases.

**Key words:** TGF-beta, cellular fibronectin, granulation tissue, actin isoforms

### The myofibroblast: definition

The myofibroblast has been initially identified by means of electron microscopy in granulation tissue of healing wounds as a modulated fibroblast exhibiting features of smooth muscle (SM) cells, such as bundles of microfilaments, with dense bodies scattered in between, and gap junctions<sup>1</sup>. The presence of myofibroblasts has successively been described in practically all fibrotic situations characterized by tissue retraction and remodeling<sup>2</sup>. The work of many laboratories has contributed to define this cell morphologically, by showing that its contractile structures are represented by stress fibers, and biochemically, by showing that stress fibers express contractile proteins typical of SM cells, particularly of vascular SM cells, such as  $\alpha$ -SM actin<sup>3</sup>. Presently it is accepted that the myofibroblastic modulation of fibroblastic cells begins with the appearance of the protomyofibroblast, whose stress fibers contain only  $\beta$ - and  $\gamma$ -cytoplasmic actins and evolves, but not necessarily always, into the appearance of the differentiated myofibroblast, the most common variant of this cell, with stress fibers containing  $\alpha$ -SM actin<sup>4</sup>. Myofibroblasts can, according to the experimental or clinical situation, express other SM cell contractile proteins, such as SM-myosin heavy chains or desmin; however the presence of  $\alpha$ -SM actin represents the most reliable marker of the myofibroblastic phenotype<sup>4</sup>.

### Factors involved in myofibroblastic differentiation

While the modulation towards the protomyofibroblast is at present not well explored, the switch protomyofibroblast/differentiated myofibroblast has been related to

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the production by inflammatory cells, and possibly by fibroblastic cells, of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), the most accepted stimulator of myofibroblastic differentiation<sup>5</sup>. The action of TGF- $\beta$ 1 depends on the local presence of the cellular fibronectin splice variant ED-A<sup>6</sup>. Thus, myofibroblast differentiation is regulated by both a cell product and an extracellular matrix component. Recently, the mechanisms by which endothelin-1<sup>7</sup> and thrombin<sup>8</sup> are able to promote myofibroblast induction have been explored. Moreover it is more and more accepted that mechanical factors play an important role in both transitions<sup>9</sup>. An increasing number of patients are being treated with growth hormone (GH) for the enhancement of body growth but also as an anti-aging strategy. Interestingly, it has been shown recently that GH inhibits TGF  $\beta$ -induced myofibroblast differentiation, resulting in a reduction in fibroblast contractile activity; in transgenic mice overexpressing GH, excisional wound closure is strongly delayed<sup>10</sup>. The question concerning the reversibility of the myofibroblastic differentiation is important, particularly for the treatment of diseases involving myofibroblasts. We can assume that fibroblasts remaining in granulation tissue after the epithelial defect is closed have reverted to a more quiescent, non-contractile phenotype lacking the microfilament bundles which were present during the contractile phase of healing. However, until now, this positive and negative modulation of the myofibroblast phenotype has not been clearly shown in vivo, even if in vitro, it seems possible to revert the myofibroblast phenotype. It is also conceivable that the residual fibroblasts represent a subpopulation of cells which failed to acquire a myofibroblast phenotype during healing and thus survive, while the myofibroblastic cells which appeared during healing represent terminally differentiated cells undergoing apoptosis during the resolution phase.

### Role of the myofibroblast in wound contraction (role of $\alpha$ -SM actin)

Recently it has been shown that  $\alpha$ -SM actin participates importantly in force production by the myofibroblast both in vitro, using models involving fibroblasts cultured on flexible substrates or within floating and attached collagen gels<sup>11</sup>, and in vivo, using experimental wound healing in the rat<sup>12</sup>. Indeed cells expressing this protein, i.e. differentiated myofibroblasts, produce a stronger retractile activity compared to protomyofibroblasts, in the absence of

any other change in contractile protein expression. Another important point is that the isometric tension produced by the myofibroblast is regulated differently compared to the reversible contraction produced by classical SM cells. While SM cell contraction is Ca<sup>++</sup> dependent and is reversible, tension production by the myofibroblast is not reversible and is regulated by a Rho/Rho kinase (ROCK)-mediated inhibition of myosin phosphatase<sup>8,13</sup>.

### Myofibroblast contraction and fibrosis

The role of the fibroblast in determining organ shape during embryonic development has been suspected for many years and is presently more and more accepted<sup>14</sup>. The most plausible mechanism of this morphogenetic action is extracellular matrix shape remodeling that in turn influences epithelial architecture. The work on the myofibroblast extends this possibility to adult tissues and gives new indications on the possible mechanisms for this action. The correct repair of connective tissue in a given organ, requires the proper reconstitution of its support function, and an appropriate tensile strength must be recreated.  $\alpha$ -SM actin expressing myofibroblasts not only promote contraction but also synthesize elevated levels of both extracellular matrix components and matrix degrading proteases. The persistence of myofibroblasts within a fibrotic lesion leads to excessive scarring with the functional impairment of the affected organ. Thus, the interactions between the myofibroblast and its surrounding extracellular matrix play an important role in the resultant mechanical properties of the connective tissue<sup>9</sup>.

It is well known that many epithelial tumors are characterized by the local accumulation of connective tissue cells and extracellular material; this phenomenon has been called the stroma reaction. One of the cellular components of the stroma reaction is the myofibroblast. Myofibroblasts interact with epithelial cells and other connective tissue cells and may thus control such phenomena as tumor invasion and angiogenesis<sup>15-17</sup>. On this basis, the myofibroblast may represent a new important target of antitumor therapy.

### Origin of the myofibroblast

Myofibroblasts of wound tissue have been assumed to originate from local recruitment of fibroblasts in the surrounding dermis and subcutaneous

tissue<sup>18</sup>. This is supported by the presence of many fibroblasts showing proliferation marker-positive nuclei at the periphery of the wound. Another possible source of myofibroblasts is represented by pericytes or vascular SM cells around vessels. During renal fibrogenesis, it has been shown that fibroblasts arise in large numbers by local epithelial-mesenchymal transition<sup>19</sup>. However, further work is necessary to clearly define the process of epithelial-mesenchymal transition (or transdifferentiation), and to evaluate its role during pathological tissue repair. In the last years, evidence has been provided suggesting the existence of circulating precursor cells, called fibrocytes, that migrate into the wound and contribute to the formation of the myofibroblastic population of granulation tissue<sup>20</sup>. Finally, it has been shown that progenitor cells located in the dermal sheath that surrounds the outside of the hair follicle, not only maintain and regenerate the dermal papilla, but also can perform important functions in the repair of skin dermis after injury<sup>21</sup>. Furthermore, interesting data demonstrate the potential usefulness of these follicle dermal cells in the construction of human skin equivalents and skin substitutes<sup>22</sup>. It should be stressed, however, that, irrespective of the origin of the lesion, the major source of fibroblasts in granulation tissue is recruitment by chemotaxis and subsequent migration from the surrounding connective tissue.

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## Conclusion

During the last years important advances have been made in the understanding of several aspects of myofibroblast biology. It remains to apply the new biological knowledge to the modulation of myofibroblast activities that regulate the evolution of fibrotic disease. Several avenues are practicable, such as influencing myofibroblast apoptosis and/or replication or collagen and/or proteolytic enzyme production by myofibroblasts.

Recently, the selective inhibition of  $\alpha$ -SM actin incorporation into stress fibers by the administration of its N-terminal sequence AcEEED has been shown to result in reduction of the tension exerted by cultured myofibroblasts on their substratum coupled with a significant decrease of collagen type I synthesis by the same cells<sup>23</sup>. Moreover this sequence, administered as a fusion peptide (FP) with a cell penetrating sequence, produces a significant reduction of the contractile capacity of granulation tissue strips after endothelin-1 stimulation and a significant delay of wound contraction in rat wounds splinted for 10 days and treated for the last 3 days with the FP<sup>23</sup>. We hope that further work in this direction as well as in other aspects of myofibroblast biology will eventually result in efficient pharmacological tools improving the evolution of such diseases as hypertrophic scars, and liver, kidney or pulmonary fibrosis.