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HISTORY OF INFLAMMATION

Giulio Gabbiani and the discovery of myofibroblasts

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Abstract
Myofibroblasts, specialized fibroblasts expressing the protein alpha-smooth muscle actin, are instrumental in wound contraction during normal wound healing. Tissue shortening is then stabilized by the synthesis of extracellular matrix, collagen in particular. Alpha-smooth muscle actin within myofibroblasts becomes organized in filamentous bundles, called stress fibers, that allow the retractile movement producing wound contraction. During hypertrophic scarring, skin deformations depend on the inappropriate action of these stress fibers that for unknown reasons persist even after the epithelialization of the wound. This historical review article is dedicated to the reconstruction of the discovery of this cell by the Italian scientist Giulio Gabbiani.

Keywords Inflammation · History of medicine · Myofibroblasts · Wound healing

Biographic sketch

Giulio Cesare Gabbiani (Fig. 1) was born on March 13, 1937, in Cremona, Italy, son of Alceste and Rosita (Grisi) Gabbiani. Giulio obtained the MD degree in 1961 at the University of Pavia (Italy) and the PhD degree in 1965 at the University of Montreal (Canada). He was also Doctor in Philosophy, University of Montreal, 1965. He was Assistant Professor at the Institute of Experimental Medicine and Surgery, University of Montreal, Research Associate at the Department of Pathology of Harvard Medical School (1967–1968), Assistant Professor at the Department of Pathology and Immunology of the University of Geneva (Switzerland) and then full Professor in the same Department since 1990, where he is now Emeritus Professor. He received Doctor in Medicine (honorary) from University of Goteborg (Sweden), 1991.

In the context of emerging work on cytoskeleton morphology and function, the ultrastructural observation made by Professor Gabbiani in 1971 showed that during granulation tissue evolution, fibroblasts acquire smooth muscle cell features, such as the presence of cytoplasmic microfilament bundles, allowing the proposition that these cells are the source of the force producing wound contraction, and connective tissue retraction during the fibrotic phenomenon.

The scientific interests of Professor Gabbiani include also soft tissue remodeling during development and pathological situations, such as wound healing and organ fibrosis as well as arterial smooth muscle adaptation during development and diseases, e.g., atheroma formation and restenosis.

The discovery of myofibroblasts

In 1971, Gabbiani published the first evidence of the presence of modified fibroblasts with smooth muscle-like features in the granulation tissue of healing wounds and proposed for this cell the name of myofibroblasts [1]. In this work, the authors described at ultrastructural level the presence of cytoplasmic microfilament bundles, analogous to the stress fibers present in cultured fibroblasts, and peripheral focal adhesion. As Gabbiani pointed out: "The contractile activity of myofibroblasts is a crucial factor for connective tissue remodeling during wound healing and creates a stressed matrix, which in turn promotes myofibroblast differentiation in a mechanical feedback mechanism" [2].

Myofibroblasts disappear when a wound close, mainly through apoptosis, or revert to quiescent fibroblasts [3]. Mechanisms that lead to myofibroblast apoptosis in

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Fig. 1 A portrait of Giulio Gabbiani

physiologic wound repair remain unclear; it has been suggested that cross-linking of a contracted extracellular matrix shields myofibroblasts from biomechanical stress and that loss of mechanical tension may induce myofibroblast apoptosis [4]. Inappropriate delay of apoptosis, and thus increased survival of myofibroblasts activated during the healing process, is a factor which leads to excessive scarring such as that seen in hypertrophic scars or fibrosis.

In 2001, Gabbiani demonstrated that focal adhesion size and composition are modulated by intracellular and extracellular factors that regulate the myofibroblast phenotype [5]. In detail, the actin bundles terminate at the myofibroblast surface in the fibronexus, a specialized adhesion complex using transmembrane integrins to link intracellular actin with extracellular fibronectin domains [5]. Transmission of high myofibroblast contractile activity to the extracellular matrix during wound contraction seems to be the major function of the fibronexus. Otherwise, it is to note that fibroblasts lack contractile microfilaments and stress fibers observed in myofibroblasts.

In 1978, Gabbiani confirmed the existence of gap junctions connecting myofibroblasts to each other similar to smooth muscle cells, suggesting that myofibroblasts form multicellular contracting units during granulation tissue contraction [6].

In 1986, Gabbiani produced a specific antibody against alpha-smooth actin, the isoform expressed by vascular smooth muscle cells and demonstrated that myofibroblasts express alpha-smooth actin [7]. Incorporation of alpha-smooth actin into the cellular stress fibers significantly augments the contractile activity of myofibroblasts [8].

Accordingly to Gabbiani, a change in the connective tissue microenvironment is responsible of the differentiation of a fibroblast into a proto-myofibroblast characterized by the presence of stress fibers expressing beta and gamma actin (Fig. 2) [9]. Finally, the proto-myofibroblast differentiates into myofibroblast, characterized by the expression of alpha-smooth actin, desmin, smooth muscle myosin heavy chains, and ED-A fibronectin (Fig. 2). Fibroblasts cultured on a plastic substrate with fetal calf serum acquire proto-myofibroblastic characteristics.

Myofibroblasts containing both fibroblasts and smooth muscle cell lineages are characterized by positive expression of stromal cell type markers such as prolyl 4-hydroxylase [10, 11], integrin $\alpha_2\beta_1$, vimentin, and CD34, and by positive expression of smooth muscle cell markers such as α-smooth muscle actin, smooth muscle myosin, calponin, and α1-integrin [12].

Gabbiani demonstrated that heparin and transforming growth factor-beta1 (TGF-β1) induce alpha-smooth muscle actin expression in granulation tissue myofibroblasts [13, 14]. Moreover, the fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by TGF-β1 [15]. Both TGF-β1 and ED-A fibronectin in the presence of mechanical stress promote the terminal differentiation of proto-myofibroblasts into myofibroblasts. Inhibition of the interaction between the ED-A fibronectin and the cell surface blocks TGF-β-induced myofibroblast differentiation [15]. TGF-β1 enhances the expression of alpha-smooth actin, the assembly of stress fibers and the formation of fibronexus adhesion complexes [5, 16]. Moreover, TGF-β1 contributes to fibrosis by the direct activation of myofibroblast synthesis of fibronectin and laminin, collagen types I, III, IV, and VI.

Other cytokines involved in myofibroblast differentiation include platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), monocyte chemotactic protein 1 (MCP1), tumor necrosis factor alpha (TNF-α), and interleukins-1β and -6 (IL-1β and IL-6) [17].

Pathological correlates

In the case of chronic inflammation, myofibroblasts continue to produce extracellular matrix resulting in fibrotic tissue, which is also abundant in certain tumor types. Myofibroblasts are typical of contracting granulation tissue in an open wound and of fibrotic contractive diseases, including scleroderma, liver and kidney fibrosis. In scleroderma, myofibroblasts are the principal stromal cells involved in the excessive extracellular matrix deposition. Myofibroblasts derive from other cells, including vascular smooth muscle cells, pericytes, endothelial cells (Fig. 3) [18], and from epithelial cells in the skin (Fig. 3) [19].

Myofibroblasts, absent from normal liver, originate from activated hepatic perisinusoidal cells after experimental liver injury [20]. In any case, portal fibroblasts acquire a myofibroblastic phenotype [21]. As Gabbiani pointed out "Myofibroblasts play a major role in the formation of septa observed in liver fibrosis and cirrhosis, and are also a major component of the stroma reaction which develops around

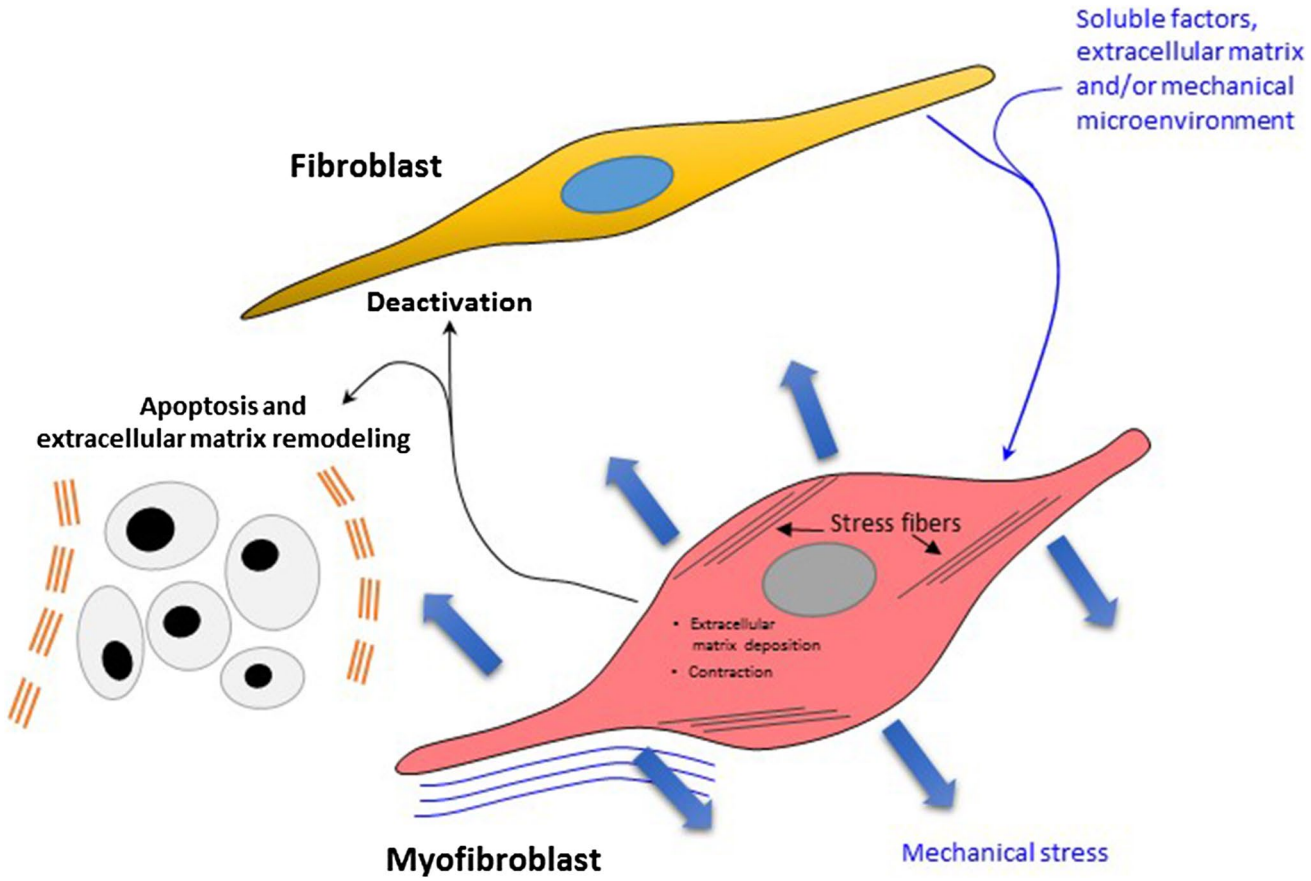

Fig. 2 Evolution of the myofibroblast phenotype. The myofibroblastic modulation of fibroblastic cells begins with the appearance of the proto-myofibroblast, whose stress fibers contain only β- and γ-cytoplasmic actins and evolve into the appearance of the differentiated myofibroblast, with stress fibers containing α-smooth muscle actin. Soluble factors, extracellular matrix components, and/or the mechanical microenvironment are involved in myofibroblastic differentiation. The myofibroblast can disappear by apoptosis

hepatocellular carcinoma. In addition, myofibroblasts deriving from hepatic stellate cells can play a role in the capillarization of the sinusoids and in the neovascularization of the tumor which facilitates the formation of metastasis" [22].

In renal fibrosis, it is not clear the myofibroblast origin, even if an altered renal microenvironment is mainly responsible for its differentiation, and cross-talk between epithelial cells and fibroblasts take place. During renal fibrosis, more than a third of myofibroblasts originate from tubular epithelial cells at the site of injury [23].

Myofibroblasts are not normally found in the healthy myocardium, but are the most prevalent cell type in the infarct scar and are the main effectors of fibrogenesis [24]. Cardiac myofibroblasts are also highly proliferative, and those isolated from infarcted myocardium exhibit a higher rate of proliferation than cardiac fibroblasts from remote areas [25]. In addition to their key role in cardiac healing, persistence of myofibroblast activation can drive pathological fibrosis, resulting in arhythmias, myocardial stiffness and progression to heart failure.

Myofibroblasts are the main components of the stromal reaction to several epithelial tumors and contribute to the organization of tumor microenvironment, which plays a critical role in tumor progression [9, 26]. The secretion of pro-inflammatory cytokines by tumor fibroblasts influences the recruitment of other inflammatory cells, which amplify the tumor inflammatory reaction and facilitate tumor cell invasion and metastasis. Myofibroblasts might support cancer-invasive properties through facilitation of the initial attachment and also through infiltrative movement of cancer epithelial cells. Moreover, myofibroblasts are capable of remodeling extracellular matrix by secreting metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [27].

Concluding remarks

Since their first description, our knowledge about myofibroblast biology has increased greatly, and this cell has been implicated in many pathological situations in addition to

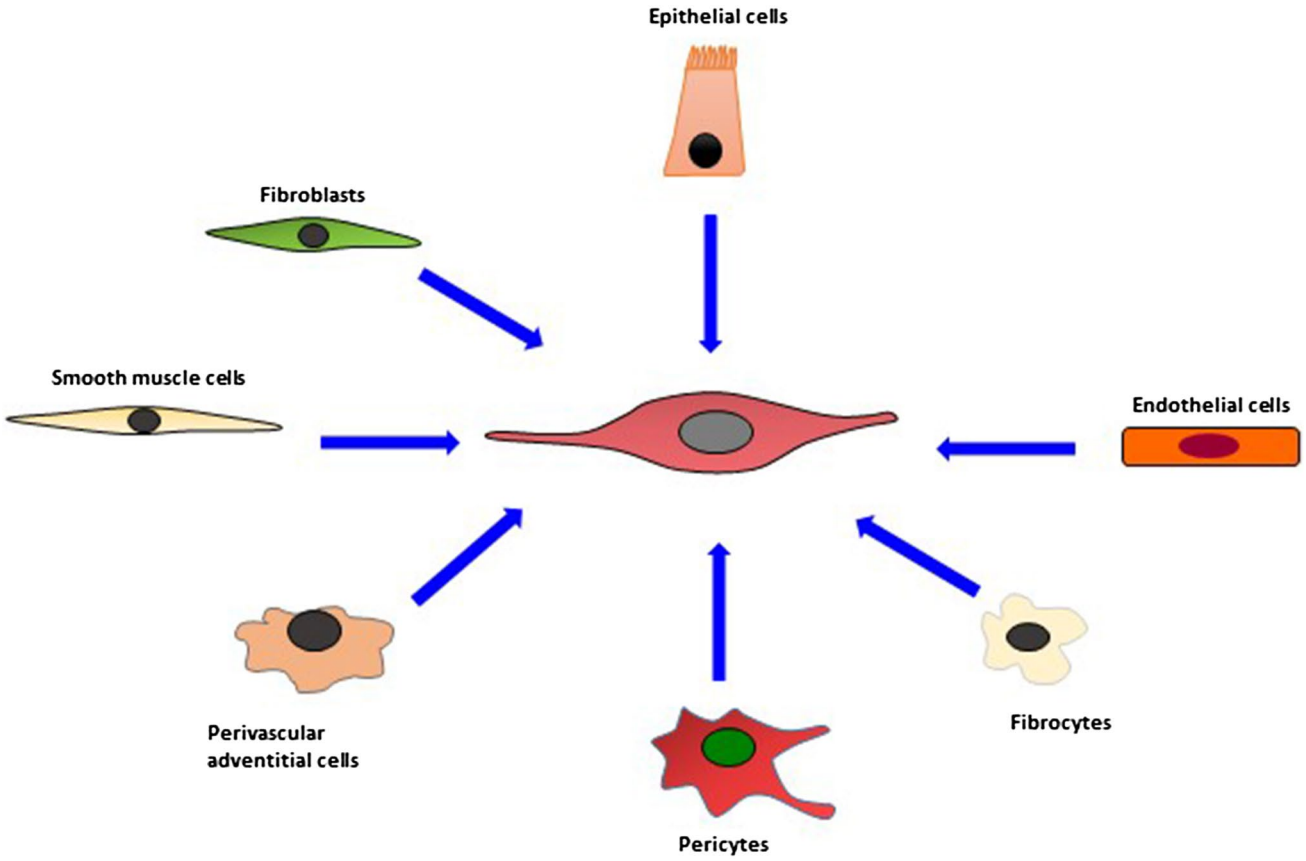

Fig. 3 Myofibroblasts can differentiate from a variety of precursor cell types, including epithelial cells, endothelial cells, fibrocytes, pericytes, perivascular adventitial cells, smooth muscle cells, and fibroblasts

their role in normal wound repair. In fact, myofibroblasts play a crucial role in organ fibrosis and tumorigenesis and are not a single cell population but a mixture of different cell populations with different functions.

As Gabbiani pointed out "Myofibroblast origin and its tissue environment should be considered when planning new therapeutic strategies that aim at decreasing myofibroblast number or activity" [8].

Drug targeting subpopulations of myofibroblasts will be a new approach to specifically modify the disease-inducing functions of myofibroblasts. Anti-fibrotic therapies may interfere with the extracellular chemical and mechanical factors leading to myofibroblast formation, or may interfere with intracellular signaling pathways involved in the modulation of myofibroblast differentiation, or may induce myofibroblast regression and/or apoptosis [28].

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