



Myofibroblasts: In health and diseases

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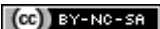
ABSTRACT

Myofibroblast are mesenchymal cells, these cells are found in the stroma of many tissues of the body. They are usually spindle shaped and also having different type of morphology. MFs possess bundles of actin microfilaments (stress fibers) containing α -smooth muscle actin (α -SMA). A soluble factor transforming growth factor- β 1 is considered a major growth factor that directly promotes MF development by inducing α -SMA expression. Modulation of fibroblast to MF represents a key event in wound healing process. They are also found in various conditions like pathological remodeling, fibrosis, oral submucous fibrosis and in stroma of invasive and metastatic carcinoma odontogenic cysts/tumors and odontogenic cysts/tumors. This review describes the nature of Myofibroblast in health and various diseases in relation to the oral cavity.

Key words: Fibroblasts, fibrosis, mesenchymal cells, myofibroblasts, stroma, wound healing

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INTRODUCTION

Myofibroblast are the component of mesenchymal origin present in stroma. These cells are spindle-shaped in appearance also having the various shapes.¹ Several phenotypes include — noncontractile fibroblasts, contractile myofibroblasts (MFs), and intermediate phenotypes — protomyofibroblasts (PMF).¹ Myofibroblasts were originally identified in granulation tissue as modified fibroblasts exhibiting bundles of microfilaments with dense bodies scattered in between, and gap junctions. Gabbiani pioneered in this field and first termed these cells as MFs.² MFs are present in organs with a high remodeling capacity such as kidneys, lungs and in the oral cavity, periodontal ligament, gingiva. They are contractile cells that play an important role in morphogenesis, organogenesis, inflammation, wound healing and fibrosis in most organs/tissues.³

Fibroblasts are metabolically active cells, which play a major in regulating and maintaining extracellular homeostasis and when activated after tissue injury, It is generally accepted that fibroblasts-MFs differentiation represents a key event during the physiological reconstruction of connective tissue after injury. MFs play a major role in the inflammatory response as they are producers of both chemokines and cytokines and are capable of augmenting or downregulating the inflammatory response, by secretion of inflammatory mediators.¹ Thus, lymphocytes, mast cells and neutrophils along with the MFs participate in immunological and inflammatory reaction.

When activated, MFs also expresses adhesion molecules such as intercellular adhesion molecule, vascular cell adhesion molecule, and neural adhesion molecules. Finally, they produce ECM molecules such as collagen type I, glycosaminoglycans, tenascin, and fibronectin (FN), which are the part of its structure, growth, differentiation and wound healing function. Thus, MF are responsible for wound contraction, fibrosis, scarring and regulation of inflammatory reaction. Their altered number and function have been implicated in diseases with increased ECM deposition and resultant fibrosis.⁴ In addition, MF participates to the process called stroma reaction and promotes cancer progression by creating a stimulating microenvironment for epithelial tumor cells.^{5,6}

MYOFIBROBLAST DIFFERENTIATION

After tissue injury fibroblasts become activated and acquire migratory phenotype and repopulate the damaged tissue.⁷ This change occurs in response to

changes in the composition and organization ECM and to cytokines that are locally released from inflammatory and resident cells or from malignant epithelial cells.^{1,3}

Another important stimulus for this phenotypic transition is the change of the mechanical microenvironment. Fibroblasts in intact tissues are generally stress shielded by the cross-linked ECM; this protective structure is lost in the continuously remodeled ECM of injured tissue.³ In response to such mechanical challenge, it first acquires contractile bundles (stress fibers) that are composed of β and γ cytoplasmic actins,^{2,8} and generate small traction forces.⁷ Tomasek et al. proposed the term “PMF” to discriminate such activated fibroblasts from quiescent fibroblasts, which are devoid of the contractile apparatus in intact tissues.⁹ PMF is a stable phenotype, representing an intermediate step, and it proceeds toward differentiation. They are connected to each other by N-cadherin type adherens and gap junctions.³ These cells are also in contact with ECM proteins at sites of integrin containing cell-matrix junctions, called “fibronexus” — a transmembrane complex made up of intracellular contractile microfilaments and the ECM protein — FN.^{1,3}

With increasing stress in the ECM, resulting from their own remodeling activity, “PMF” further develop into “differentiated MFs” by neo-expressing α -SMA — most widely used MF marker.⁷ Expression of α -SMA is precisely controlled by combined action of growth factor — transforming growth factor (TGF)- β 1, and ECM protein — FN splice variant ED-A FN and mechanical environment.⁹

MFs also expresses α and β integrins that are part of adhesion mechanism of MFs to matrix proteins. It is considered as a mature MF. However, it is important to note that many cells including myoepithelial, endothelium, pericytes and smooth muscle cells also are α -SMA positive. Despite this MF is considered as a fibroblast-like α -SMA positive cell with no further characteristic features.⁹ Thus, ultrastructural features that discriminate MFs from quiescent fibroblasts in tissues are:⁷

1. Bundles of contractile microfilaments (stress fibers) with dense bodies;
2. Intercellular adherence and gap junctions;
3. Extensive cell to matrix attachment sites — “fibronexus”.

ORIGIN

Myofibroblasts may have heterogeneous origins.⁷ MF is typically considered to be activated fibroblasts. Depending on the type of tissue to be

remodeled, MF precursor cells are recruited from different sources.³ The main progenitors of MF appear to be the locally resident fibroblasts.¹⁰ Other mesenchymal cells that serve as precursors are pericytes and smooth muscle cells from vasculature, (during vessel repair) and seem to play a role during fibrosis in scleroderma.⁴

In addition bone marrow (BM) derived leukocytes known as fibrocytes,¹¹ having fibroblasts characteristics have been suggested to represent an alternative source for MF during skin wound healing and in liver, lung, and kidney fibrosis^{3,6} as well as in the stromal reaction to epithelial tumors.³ Another type of nonhemopoietic precursor cells originating from BM are mesenchymal stem cells, which are also suggested to participate in tissue repair.⁵

Finally, epithelial- and endothelial-to-mesenchymal transition (EMT), a process by which differentiated or malignant epithelial and endothelial cells undergo a phenotypic conversion that gives rise to the matrix producing fibroblasts and MFs, is increasingly recognized as an integral part of tissue fibrogenesis after injury, particularly in the kidney and also in squamous cell carcinoma.^{12,13} Overall these cells represent alternative sources of MFs when local fibroblasts are not able to satisfy the tissue's requirement.^{3,6}

The components of ECM of MF populated tissue that can potentially be used as molecular markers are:⁷

1. Collagen of types I, III, IV;
2. FN splice variant ED-A FN (most reliable marker);
3. Another component of MF-ECM — glycoprotein tenascin-C which is associated with tissue repair phenomenon.

Tenascin-C appears to attract fibroblasts and promote their differentiation into MF in injured tissue and at the tumor invasive front. Expression of specific proteins, transmembrane cell-cell adhesion proteins that are linked to cytoplasmic actins is a new strategy to target and identify MF.⁷

ROLE IN NORMAL WOUND HEALING

The process of wound healing is a highly orchestrated event. Various cytokines and growth factors have a role in wound healing and scarring. MFs play a crucial role in wound healing that proceeds in three interrelated dynamic phases.^{1,6,8}

Inflammatory phase: Following injury damage to capillaries, the trigger formation of a blood clot, consisting of fibrin and FN. This matrix will fill the lesion. Platelets are present in the blood clot release chemokines, which participate in recruitment of

inflammatory cells (neutrophils/macrophages), fibroblasts and endothelial cells at the site of injury.

Proliferative phase: Proliferation of endothelial cells resulting in the active formation of new capillaries contributes to the proliferation of fibroblasts. Such fibroblasts start to generate granulation tissue components such as hyaluronic acid, collagen, and FN. In this granulation tissue activation of few fibroblasts result in MF differentiation. These MF cells synthesize and deposit ECM components which will replace the provisional matrix.

Major source of fibroblasts in granulation tissue is recruitment by chemotaxis and subsequent migration from surrounding connective tissue.² These cells, because of its contractile properties help in the contraction and in the maturation of the granulation tissue. This involves gradual replacement of MFs N-cadherin by OB-cadherin (cadherin-11).⁹

Scar formation and wound contraction It involves progressive remodeling of the granulation tissue. During this process, proteolytic enzymes essentially matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases) play a major role.¹⁴ At the end of tissue repair, the reconstructed ECM again takes over the mechanical load and MFs disappear by massive apoptosis.⁹ α -Smooth muscle actin in the stress fibers augments the contractile activity of fibroblasts cells and hallmarks the contraction phase of connective tissue remodeling.⁷ α -SMA participates in force production.⁹

MF develops the capacity of producing long lasting tension, which is regulated by a RHO/RHO kinase mediated inhibition of myosin light chain phosphatase.¹⁵ Smooth muscle cell contraction/relaxation is Ca^{++} dependent and is reversible, whereas tension produced by the MF is not reversible.² MF generated tension is instrumental for tissue remodeling and deformation during fibrocontractive diseases.³

PATHOLOGICAL REPAIR

The fate of activated MFs in the injured tissues depends upon whether normal healing occurs or progress to end-stage fibrosis. TGF- β and PDGF are the key factors in the fibrosis. Pathological wound healing can be encountered in a variety of disease states. Normally after completion of repair, MFs disappear by apoptosis. However, persistent presence of MFs stimulates dysfunctional repair mechanism, leading to excessive contraction and ECM secretion resulting in fibrosis. This mechanism has been implicated in hypertrophic

scar, scleroderma, palmer fibromatosis of Dupuytren's disease as well as in heart, lung kidney fibrosis.³ It is proposed that in fibrosis, MFs acquire an immune privileged cell phenotype, which helps them to evade apoptosis and permits their uninterrupted accumulation.¹⁶ MFs appear to play a significant role in promoting ECM deposition, release of inflammatory mediators, and epithelial injury, all of which are considered to be key factors in perpetuating the cycle of injury and fibrosis.^{3,7}

ROLE IN ORAL SUBMUCOUS FIBROSIS

Myofibroblasts are key cellular mediators in various fibrotic disorders.¹⁷ Oral submucous fibrosis (OSMF) represents an abnormal healing process of oral mucosa after chronic sustained injury resulting in scarring and fibrosis, as demonstrated by increased incidence of MFs, in a recent study. TGF- β is a key molecule related to imbalance between collagen deposition and degradation in OSMF in response to arecoline challenge. Angadi et al. suggested that MFs could be used as a marker for evaluating the severity of OSMF. In addition, they also proposed that as MFs are responsible for producing a variety of factors that are involved in the fibrotic process; they could be the key link in the pathogenesis of OSMF. Interfering with its development/recruitment could provide a therapeutic approach to combat fibrosis.¹⁸

ROLE IN SQUAMOUS CELL CARCINOMA

Myofibroblasts are predominant cell type in different primary and metastatic epithelial tumors. Presence of MF phenotype has not been demonstrated in lesions with epithelial dysplasia.¹⁹ Many epithelial tumors are characterized by the local accumulation of connective cells and ECM; this phenomenon has been called the stroma reaction.^{2,5} Fibroblasts contributing to the tumor stroma have been termed peritumoral fibroblasts, reactive stroma, carcinoma associated fibroblasts.⁶ At the interface between stromal fibroblasts and oral carcinoma invasive front region, some show change to MF phenotype and lie in close proximity to tumor Island. This has led to the concept that these stromal MFs might originate by epithelial-mesenchymal transition of tumor cells.¹³ Squamous carcinoma cells may directly induce an MF phenotype in primary fibroblasts through the

secretion of TGF- β 1.¹⁴ In general stromal reaction to epithelial neoplasm is marked by the appearance of MFs. Stromal MFs are known to remodel the ECM and helps in its degradation by secretion of matrix metalloproteinase, thereby promoting the invasive growth of epithelial lesions.⁶

Myofibroblasts influence tumor progression and invasion. Apart from stimulation of fibroblast-MF differentiation, TGF- β increases synthesis of ECM protein, including collagen. In vitro studies have shown significantly increased invasion associated with increased collagen activity.²¹ Stroma cells may not only be implicated in demoplastic tissue remodeling but even contribute actively to tumor progression.⁶

ROLE IN ODONTOGENIC LESIONS

The presence of MFs in odontogenic lesions is not thoroughly investigated. Vered et al.²² suggested that increased number of MFs appear to be directly correlated with aggressive biological behavior. Roy and Garg²³ found increased number of stromal MFs in keratocystic odontogenic tumors in comparison to odontogenic keratocyst and correlated it with its aggressive biological behavior and increased tendency for recurrence.

SUMMARY

Myofibroblasts are unique subpopulation of fibroblasts cells, phenotypically intermediate between SM cells and fibroblasts. Fibroblast/ MF transition is accepted not only as a key event in the formation of granulation tissue during wound healing or fibrotic changes, but also during evolution of the stroma reaction in cancer. Local resident fibroblasts are a major source of MFs. However, their development follows orderly sequences of events. TGF- β 1 is the main stimulus for fibroblast/MF modulation. MFs are present in many tissues and play an important role in various organ diseases. MFs is instrumental in stroma reaction of epithelial tumors and considered to create a stimulating microenvironment for transformed cells. As stroma cells represent an important target of anticancer treatment. Future studies in this field may provide new avenues on planning devices/tools for treatment strategy and improving the evolution of fibrotic diseases and cancer.

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