Review Article

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Mysterious myofibroblast: A cell with diverse origin and multiple function

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ABSTRACT

Myofibroblasts are one of the most controversial cells in recent times. Ever since its first discovery, numerous discussions have been done on its illusive nature and functions. They are commonly considered as smooth muscle like fibroblasts. Their presence and distribution in normal and pathological conditions are still not clear since they are difficult to identify with the routine histological techniques. Recent studies have shown their ubiquitous presence in the body tissues hence suggesting their important role in both physiological functioning and pathological conditions. This review discusses briefly the cell in terms of its definition, possible precursors; mechanism involved in its modulation, most importantly how to differentiate it from its nearest counterparts such as fibroblasts and smooth muscle cells and finally its fundamental role in physiology and pathology.

KEY WORDS: Alpha-smooth muscle actin, desmoplasia, fibronexsus, myofibroblasts, transforming growth factor-β, wound healing

INTRODUCTION

The word "myofibroblast" has gained lot of attention in recent times though it remains equally controversial. Myofibroblasts are considered as distinct since they express features of two separate cells within a single cell phenotype and controversial since their presence, role in health and disease is still not clear [1]. Most of the connective tissues in the body are under some sort of mechanical tension, even during the rest phase. The fact that under normal conditions soft connective tissues around relaxed musculature does not sag provides evidence for it. Actually, a resting tension is built into the anatomy of these tissues which prevents such slackness. The cellular and molecular basis of this balance and the mechanisms acting to reestablish it after tissue injury are not very clear [2]. The advent of mechano regulating cell like myofibroblast has offered better understanding of such mechanism [3].

Originally, modified fibroblasts with smooth muscle-like features were first observed in granulation tissue of healing wounds suggesting their role in the production of the contraction required for wound healing [4]. Currently, myofibroblasts have been also identified in normal tissues, particularly in locations where there is a necessity of mechanical force development and which require constant remodeling in response to stress [5].

Of late, myofibroblasts are linked to numerous pathological conditions which are associated with fibrosis [4]. Myofibroblasts are also one of the cancer-induced host cells of the tumor microenvironment. The cross-talk between cancer cells and myofibroblasts is largely thought to influence tumor invasion and metastasis [6].

The current review on myofibroblasts highlights briefly regarding the origin of myofibroblasts, characteristic features

of myofibroblast cells, mechanism, and factors involved for their differentiation from progenitors and finally the role of myofibroblasts in physiological and pathological conditions.

DEFINING THE CELL *MYOFIBROBLAST* AND THEIR ORIGIN

The cell-myofibroblast was first defined by Gabbiani *et al*. [7]. Myofibroblasts are modulated fibroblast exhibiting features of smooth muscle cells, such as bundles of microfilaments, with dense bodies scattered in between, and gap junctions [8]. An appropriate definition for the granulation tissue and tumor stroma myofibroblast would be derived using electron microscopy, i.e. A cell with prominent rough endoplasmic reticulum, modestly developed peripheral myoflaments with focal densities, fibronexus junctions, sometimes a Golgi apparatus producing collagen secretory granules, and gap junctions [1,8].

Myofibroblasts of wound tissue have been assumed to originate from locally residing of fibroblasts in the surrounding dermis and subcutaneous tissues by a process called transdifferentiation [4,9]. Myofibroblasts may also originate from progenitor stem cells in the neural crest [10]. In the recent years, evidence has been provided suggesting the existence of bone marrow-derived circulating cells, called CD34 fibrocytes as precursors of myofibroblasts [11]. Embryonic and mesenchymal stem cells, few local cell types such as pericytes, endothelial cells, and even malignant epithelial cells have been proven to be the source of myofibroblasts [12].

MECHANISM OF MYOFIBROBLAST CELL FORMATION FROM RESIDENT FIBROBLASTS

Under normal circumstances, fibroblasts do not exhibit actin-associated cell-cell and cell matrix contacts. Fibroblasts in intact tissue are generally stress-shielded by the cross-linked extracellular matrix (ECM) however, during any injury ECM undergoes remodeling and protection will be lost. In response to mechanical stress, fibroblasts acquire contractile stress fibers but stain negatively with alpha-smooth muscle actin (α-SMA) thus forming the "protomyofibroblast" [13]. Stress fibers are connected to fibrous ECM proteins at focal adhesions [14] [Figure 1]. Protomyofibroblast represents an intermediate step in the formation of "differentiated myofibroblast" which is characterized by *de novo* expression of α-SMA in stress fiber pattern and large supermature fibronexus adhesion complexes [14] [Figure 1]. Demonstrations on wound granulation tissue and myofibroblast culture on elastic substrates have shown that the threshold stiffness required for *de novo* expression of α-SMA in a stress fiber pattern is around 20,000 Pa [15]. High extracellular stress generated due to remodeling of the ECM [2], the presence of specialized ECM proteins like the extra domain A (ED-A) splice variant of fibronectin and accumulation of biologically active transforming growth factor-beta 1 (TGFβ-1) are the three basic necessities to generate α-SMA positive differentiated myofibroblasts [14]. Under the influence of TGFβ and mechanical tension, the α-SMA gets recruited on beta-cytoplasmic actin filaments and acquire subplasmalemmal stress fiber pattern [16]. The major pathway through which TGFβ-1 regulates expression of α-SMA in fibroblasts involves Smad signaling [12]. Other cytokines involved are platelet-derived growth factor, tumor necrosis factor-α, basic fibroblast growth factor, endothelins, granulocyte monocyte-colony stimulating factor and interleukin-6 [2,12].

At the end of tissue repair, the reconstructed ECM again takes over the mechanical load and myofibroblasts disappear by massive apoptosis. Stress release act as powerful promoter of myofibroblast apoptosis *in vivo* [14].

Myofibroblast-cell Facts

The three essential morphologic features of myofibroblast are bundles of actin filaments with interspersed dense bodies which run parallel to the long axis of the cell and located beneath the cell membrane called "stress fibers," well-developed cell to stroma attachment sites called fibronexsus and intercellular intermediate and gap junctions [17]. Under the light microscope, myofibroblasts appear as large spindle-shaped cells with vesicular elongated nuclei [Figure 2]. However, they could also be present as stellate (spider like) cells with long cytoplasmic extensions. Cells are characterized by the presence of abundant ECM [18]. Ultrastructure of myofibroblasts shows spindle-shaped cells with nuclei showing typical irregular contour and indentation, a feature thought to reflect cellular contraction, and contain finely granular chromatin and nucleoli [1,19]. Peripheral sub plasmalemmal cytoplasm consists of myofilaments of 40-80A^0 with electron dense bodies parallel to the long axis of the cell [16,18,19]. The incomplete basal lamina is usually seen around the cell [19]. Fibronexus, a transmembrane complex composed of intracellular contractile microfilaments and the ECM protein fibronectin forms focal contacts of myofibroblasts with ECM [19].

Myofibroblasts, in addition to beta and gamma-cytoplasmic actin expression that found in fibroblasts, also show α-SMA, similar to those expressed by SM cells [2]. Irrespective of

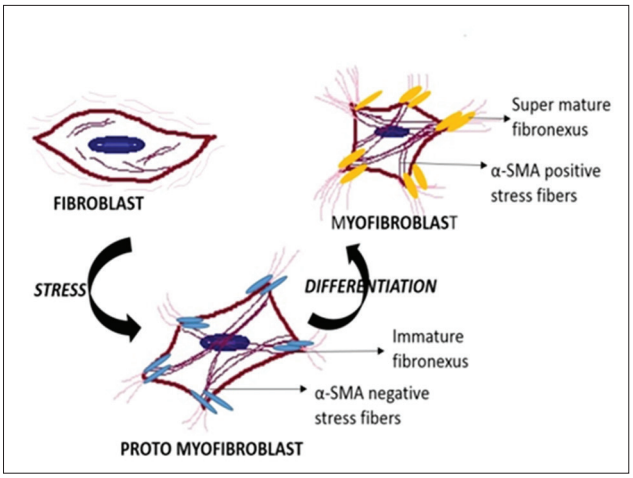

Figure 1: Two-stage model of myofibroblast differentiation from fibroblasts [12,13]

their origin, they show positivity to vimentin [20]. They also show strong positivity to ED-A splice variant of fibronectin, calponin, and muscle actin marker HHF-35 [18]. However, they lack smooth muscle myosin expression [9]. A classification system has been proposed based on immune histochemical staining of filaments of myofibroblasts. Myofibroblasts that express the only vimentin are termed V-type myofibroblasts, those that express vimentin and desmin are called VD-type, those expressing vimentin, α-SMA and desmin are VAD-type, and those that express only vimentin and α-SMA are called VA-type [19,21].

Morphologically, myofibroblasts represent an intermediate cell between fibroblasts and smooth muscle cells [19]. The differences between smooth muscle cells, fibroblasts, and myofibroblasts are very minimal [Table 1] [17,18,22].

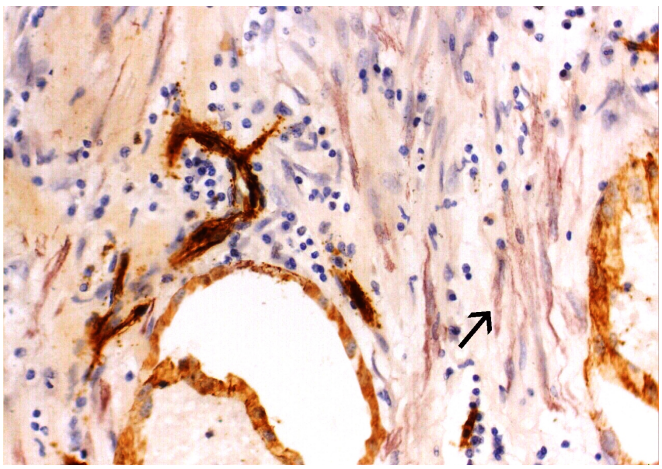

Figure 2: Tumor stroma showing myofibroblasts as a large spindle-shaped cells with cytoplasmic extensions and oval nuclei. Myofibroblast cells show alpha-smooth muscle actin (α-SMA) positivity (arrow) (double immunostaining, diaminobenzidine and VIP purple chromogen, CD34 and α-SMA monoclonal antibody, original magnification: ×40)

Myofibroblasts-functions

In skin, myofibroblasts play an important role in wound healing, epithelial growth, and differentiation of granulation tissue [19]. Wound healing is also facilitated by contractile abilities of myofibroblasts, which aids in reducing the amount of denuded surface area of wounded tissue [19]. In periodontal ligament of the oral cavity, they have a major role in attachment of teeth and tooth eruption [23]. In general, myofibroblasts participates in various physiological activities. Myofibroblasts secrete matrix molecules such as collagen and glycosaminoglycans (GAGs) into interstitial space or in basement membrane zone hence take part in maintaining structure, growth, and differentiation [9,21].

The contractile capability allows myofibroblast cells to participate in the ejection of fluid from the gastric glands, in the motility of intestinal villi and to locally autoregulate blood flow in peripheral vessels [19]. Contractile fibroblasts have been suggested to generate a beneficial mechanical stress or "tonus" in some normal tissues, such as in the alveolar septa in the lung [3]. Myofibroblasts play a major role in modulating inflammatory response since these cells are avid producers of both chemokines and cytokines [Table 2] [19]. Numerous growth factors secreted by myofibroblasts act by inducing the cell motility, proliferation, and differentiation [Table 2] [21]. They also play a fundamental role in many disease states, either through activation and proliferation or through their selective absence [19,24].

Myofibroblasts in Pathology

Pathological conditions related to myofibroblasts can be categorized as, diverse response to injury and tissue repair; proliferative conditions which mimic neoplasm; tumors of myofibroblasts and myofibroblasts as stromal reaction in certain neoplasias [17].

The first group related to myofibroblasts in granulation tissue and myofibroblasts as diverse tissue responses in burn

Table 1: Ultrastructural, morphological differences, and difference in molecular expression between fibroblasts, myofibroblasts and smooth muscle cells [13,37]

Cell features	Fibroblasts	Myofibroblasts	Smooth muscle cell
Cell shape	Bipolar/tapered	Bipolar/spindle, stellate	Bipolar/wider
Nucleus	Spindle, Smooth	Spindle, indentations	Cigar shaped
Golgi complex	Numerous	Less numerous	Scanty
RER and mitochondria	Numerous	Comparatively less numerous	Scanty
Pinocytic vesicles	Absent	Present	Numerous
Dense bodies	Absent	Present	Numerous
External lamina	Absent	Present, discontinuous	Present continuous
Attachment plaques	Present forms a component of classical FA's with only integrins	Present forms a component of super mature FAs with integrin clusters, adhesion plaques, and all actions	Present but forms attenuated FAs
Cell-cell attachment	Absent	Gap junction/adherents	Gap junction/adherents
Cell-matrix attachment	By classical (6 μm) FA's	Well-formed super mature FAs (8-30 μm)	Attenuated FAs
Collagen secretion	Predominantly Type I and Type III	Type I, Type III, Type VI and predominantly Type V	Type I, predominantly Type III and elastin
Stress fibers	α-SMA fibers are absent	α-SMA fiber in stress fiber pattern	α-SMA fibers distributed in cytoplasm
ED-A splice variant of fibronectin	Absent	Present	Absent
Glycoprotein tenascin-C in ECM	Absent	Present	Present

FAs: Focal adhesions, RER: Rough endoplasmic reticulum, ECM: Extra cellular matrix, ED-A: Extra domain A, α-SMA: Alpha-smooth muscle actin

contractures, interstitial lung fibrosis, localized and systemic scleroderma, atherosclerotic plaques, cirrhosis, sinus tracts, and ischemic ulcer beds [9,17,21]. The second group includes the fibromatoses and other soft tissue proliferations that mimic sarcomas, such as nodular and proliferative fasciitis, proliferative myositis, cutaneous fibrous histiocytoma (dermatofibroma), elastofibroma, and others [18]. The third group involves certain benign and malignant lesions of myofibroblast differentiation such as myofibromas, myofibromatosis, and myofibrosarcoma [18,21]. The fourth group concerns the stromal response to neoplasia. Many invasive and metastatic carcinomas, especially those characterized by hard consistency, retraction, and fixation to adjacent tissues, elicit a desmoplastic stromal reaction that is rich in myofibroblasts [21]. The retraction ascribed to such carcinomas has been attributed to the contractile forces generated by stromal myofibroblasts [18]. They tend to be most numerous within the young mesenchymal stroma, areas that correspond to early stromal invasion or more consistently, at the peripheral invasive front of the tumor [17].

MYOFIBROBLASTS IN TISSUE REPAIR AND DIVERSE RESPONSES TO INJURY

Myofibroblasts with α-SMA expression start appearing in granulation tissue around the 8th day of wound healing increases till 15th day and thereafter gradually starts decreasing, progressively disappear by apoptosis and no longer detected after 1 month [17]. They are most numerous in a cellular layer of granulation tissue. The coordinated contraction of myofibroblasts during this phase is believed to be responsible for wound contraction and closure [25]. The other functions of myofibroblasts are to synthesise ECM proteins, notably collagen Types I and III, tenasin, fibronectin, glycoproteins, and proteoglycans during wound repair [26]. In addition, myofibroblasts also secrete many other matrix molecules including laminin, thrombospondin, GAGs, hyaluronic acid, and heparan sulfuate, as well as matrix-modifying proteins such as matrix metalloproteinases and tissue inhibitor of metalloproteinases (TIMPs) [19,25].

Myofibroblasts in Fibrotic Diseases

The myofibroblasts act as the key features of active fibrosis by its ability to express high levels of ECM and fibrogenic cytokines.

Table 2: List of growth factors, chemokines, and inflammatory mediators secreted by myofibroblasts [19,21]

Cytokines	IL-1, IL-6, IL-10, TNF-α
Growth factors	TGF-β, CSF-1, GM-CSF, PDGF, bFGF, IGF, KGF, HGF, SCF, VEGF
Inflammatory mediators	PGE_2, Prostacyclins, HETE's, PAF

IL: Interleukin, TNF-α: Tumor necrosis factor α, CSF-1: Colony-stimulating factor-1, GM-CSF: Granulocyte monocyte-colony stimulating factor, PDGF: Platelet-derived growth factor, bFGF: Basic fibroblast growth factor, IGF: Insulin-like growth factor, KGF: Keratinocyte growth factor, HGF: Hepatocyte growth factor, SCF: Stem cell factor, VEGF: Vascular endothelial growth factor, PGE2: Prostaglandin E2, HETEs: Hydroxyleicosatetraenoic acids, PAF: Platelet-activating factor, TGF-β: Transforming growth factor-beta

They also contribute to the altered mechanical properties of affected tissues [27]. Myofibroblasts are important cell type contributing to systemic and localized scleroderma. High level of TIMP secreted by myofibroblasts plays an important role in fibrotic disease by inhibiting the collagen degradation [28]. During wound healing, myofibroblasts are usually eliminated by apoptosis at the end stages. If they persist, continued remodeling of the tissue takes place causing organ fibrosis, and collectively such conditions are called as fibrocontractive disorders. Dupuytren's contracture is a fixed flexioncontracture of the hand where the fingers bend toward the palm and cannot be fully extended. Dupuytren's contracture is caused by underlying contractures of the palmar aponeurosis. Numerous studies support that the myofibroblast is a key cell responsible for the tissue contraction in Dupuytren's disease [29].

There are also several benign proliferative lesions with different degree of myofibroblastic differentiation. Pseudosarcomatous fasciitis type of lesions with prominent myofibroblastic differentiation pose diagnostic challenge due to their close resemblance to sarcomas. Nodular fasciitis, proliferative myositis, myofibroma/myofibromatosis, fibromatosis, and myositis ossificans are to name few [18]. Benign fibrous histiocytoma, aggressive angiomyxoma, ischemic fasciitis, and elastofibroma are categorized as lesions with incomplete myofibroblastic differentiation [17].

Tumors of Myofibroblasts

These are true malignant tumors arising from myofibroblast cells. Low-grade myofibrosarcoma, inflammatory myofibroblastic tumor, infantile fibrosarcoma, intermediate grade myofibrosarcoma, and high-grade pleomorphic myofibrosarcoma are to name few. These tumors are rare to occur however has to be considered in the differential diagnosis of spindle cell tumors [30].

Myofibroblasts as Stromal Response to Carcinomas

The proliferation of tumor cells and invasion involves a complex interaction between malignant cells and its surrounding matrix, resulting in the formation of specialized tissue called tumor stroma [31]. Tumor stroma comprises of acute and chronic inflammatory cells, immune cells, blood and lymph endothelial cells, fibroblasts, and specialized fibroblasts called myofibroblasts [6,32]. Cancer and stromal cells such as myofibroblasts exchange cytokines, chemokines, growth factors, ECM proteins, and enzymes [32].

Desmoplasia, which is characterized by pronounced fibrotic response with the formation of myofibroblasts, is one of the evidence for host participation in tumor stromal reaction [33]. With regard to myofibroblasts, two patterns of stromal reactions occur in squamous cell carcinoma. One is characterized by a marked proliferation of myofibroblasts and desmoplasia, with scarce lymphocytic infiltration; this patter usually seen associated with well or moderately differentiated SSC. The other is characterized by few myofibroblasts, weak desmoplasia, and dense lymphocytic infiltration; the latter pattern tends to be

associated with moderately or poorly differentiated SSC [34]. Invasion beyond the basement membrane is necessary to evoke the myofibroblastic stromal reaction [31]. The presence of myofibroblasts has been reported in the stroma of oral [32], laryngeal [31], breast [35], cervical [36], renal pelvis [37], and bladder [38] cancers. It has been correlated with both favorable and poor prognosis by different authorities [32]. Myofibroblasts may stimulate tumor progression by stimulating the growth of cancer cells, sustaining the angiogenesis and lymphangiogenesis, inhibiting the immune mechanisms and stimulating invasion and metastasis by activating proteolysis [6,32]. Numerous experimental and clinical observations indicate that myofibroblasts produce pro-invasive signals [6,11,33,39]. De Wever *et al.* showed pro-invasive activity of myofibroblasts *in vitro* by using human colon cancer cells and myofibroblasts isolated from surgical colon cancer fragments. In 48 h cultures, the colon cancer cells invaded the collagen gels only when myofibroblasts were added [11]. The cancer-induced formation of a myofibroblast network may serve as guidance structure that directs the migration of epithelial cancer cells [11].

CANCER MANAGEMENT AND MYOFIBROBLASTS

Understanding the cross-talk between tumor cells and stromal cells has provided better opportunities for newer treatment strategies [6]. Genetic stability of host cells compared to unstable tumor cells provides a better option for therapeutic target [6]. One important issue to be discussed is whether or not routine methods of cancer management affects the myofibroblasts modulation and by inference enhance invasion and metastasis. Surgical interventions make wounds and will inevitably elucidate myofibroblast stimulation as part of the healing process. Biopsies routinely practiced for histopathological diagnosis, make a wound in the core of the tumor. In areas surrounding the biopsy track in breast cancers, cancer associated fibroblasts express higher amounts of the proinvasive proteinase than in intact parts of the tumor [11]. In terms of myofibroblasts, target could be, inductive signals for myofibroblast cells such as TGFβ, signals which upregulate α-SMA expression, ED-A splice variant of fibronectin, and fibroblast activation protein [6]. However, further detailed exploration of myofibroblasts role in tumor cell proliferation, invasion, metastasis, and molecular signaling between host cells are necessary before considering them as future therapeutic targets.

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