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Review

Myofibroblasts

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ABSTRACT

Myofibroblasts are activated in response to tissue injury with the primary task to repair lost or damaged extracellular matrix. Enhanced collagen secretion and subsequent contraction – scarring – are part of the normal wound healing response and crucial to restore tissue integrity. Due to myofibroblasts ability to repair but not regenerate, accumulation of scar tissue is always associated with reduced organ performance. This is a fair price to pay by the body for not falling apart. Whereas myofibroblasts typically vanish after successful repair, dysregulation of the normal repair process can lead to persistent myofibroblast activation, for instance by chronic inflammation or mechanical stress in the tissue. Excessive repair leads to the accumulation of stiff collagenous ECM contractures – fibrosis – with dramatic consequences for organ function. The clinical need to terminate detrimental myofibroblast activities has stimulated researchers to answer a number of essential questions: where do myofibroblasts come from, what are the factors leading to their activation, how do we discriminate myofibroblasts from other cells, what is the molecular basis for their contractile activity, and how can we stop or at least control them? This article reviews the current state of the myofibroblast literature by emphasizing their role in ocular repair and fibrosis. It appears that although the eye is quite an extraordinary organ, ocular myofibroblasts behave or misbehave just like their siblings in other organs.

1. Introduction

Myofibroblasts have first been discovered in wound granulation tissue of healing skin wounds as cells bearing the secretory features of fibroblasts (prominent endoplasmic reticulum) and contractile features similar to smooth muscle (microfilament bundles) (Gabbiani et al., 1971). Four decades ago, the identification of contractile non-muscle cells *in vivo* experimentally supported that wound closure is promoted by cells within the granulation tissue and not by collagen shrinkage, a paradigm shift that started already in the 1950's (Abercrombie et al., 1956). It is easier to find something if you know what you are looking for. Once the initial ultrastructural characterization of the contractile fibroblast, hence 'myofibroblast' was established, myofibroblasts were identified on the basis of their actin filament bundles in a number of different pathologies (Hinz et al., 2012a). It is indeed a main myofibroblast characteristic to be activated as part of a normal or dysregulated wound healing response, and to be absent from the vast majority of normal tissues (Hinz et al., 2012a). Myofibroblast research gained further momentum with the finding that myofibroblast activation is associated with neo-expression of the α-smooth muscle isoform of actin (α-SMA) and generation of the respective antibody (Skalli et al., 1986). Ocular myofibroblasts were first described in the early 1980's according to their distinct ultrastructure and contractile function in retinal detachment assays (Cleary et al., 1980; Cleary and Ryan, 1981) and a decade later in human anterior capsular cataracts using α-SMA immunostaining (Schmitt-Graff et al., 1990).

Activation of myofibroblasts in the eye occurs in response to injury with the intent to repair damaged extracellular matrix (ECM), most obvious in cornea repair following various injuries such as photorefractive keratectomy (PRK) or laser in situ keratomileusis (LASIK) (Boote et al., 2012; Garana et al., 1992; Jester et al., 1999; Myrna et al., 2009; Stepp et al., 2014). This normal repair process leads to temporary corneal haze that is caused by the transient presence of cells and disorganized ECM (Wilson, 2012). While acute repair is terminated by myofibroblast de-activation and apoptosis, concomitant with the disappearance of the mild haze (Wilson et al., 2007), continued myofibroblast activities create clinical complications and impair eye function. The excessive

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secretion and contractile deformation of collagen contribute to late haze and permanently reduced transparency in the cornea (Hassell and Birk, 2010; Jester et al., 1999; Torricelli and Wilson, 2014; Wilson et al., 2001), cornea scar formation (Gomes et al., 2012; Holbach et al., 1990), contraction of the pre- and epiretinal membranes, proliferative vitreoretinopathy (PVR) and proliferative diabetic retinopathy (PDR) (Bochaton-Piallat et al., 2000; Cleary et al., 1980; Trese et al., 1985; Walshe et al., 1992), anterior capsular cataract formation (Novotny and Pau, 1984), and primary open-angle glaucoma (POAG) (Kirwan et al., 2005). Hence, the mechanisms of myofibroblast activation and action have to become more transparent, literally.

2. What is a myofibroblast – functions, features, forbears

2.1. Myofibroblast actions and consequences

Similar to the situation in the eye, myofibroblast activation in other organs is part of the normal wound healing response that is typically terminated when tissues are repaired (Hinz, 2007). At this stage, myofibroblasts are cleared by apoptosis (Desmoulière et al., 1995; Wilson et al., 2007) or may become de-activated as suggested by an increasing number of reports (Hecker et al., 2011; Kisseleva et al., 2012; Talele et al., 2015; Troeger et al., 2012). In contrast, persistent myofibroblast activities cause accumulation and contraction of collagenous ECM, a condition called fibrosis. Organ function is severely impaired or lost in fibrosis, as described for hypertrophic scars (Gauglitz et al., 2011), scleroderma (Castelino and Varga, 2014), Dupuytren's disease (Hinz and Gabbiani, 2011; Verhoekx et al., 2013; Vi et al., 2009), liver (Forbes and Rosenthal, 2014; Liedtke et al., 2013), heart (Davis and Molkentin, 2014; Turner and Porter, 2013; Weber et al., 2013), lung (Noble et al., 2012; Sivakumar et al., 2012) and kidney (Campanholle et al., 2013; Duffield, 2014). Myofibroblasts are also main drivers in the stroma reaction against tumors and promote cancer progression by creating a stimulating microenvironment for the epithelial tumor cells (Cox and Erler, 2011; Gritsenko et al., 2012; Lu et al., 2012; Pickup et al., 2014; Yu et al., 2011). In all these pathologies, the neo-appearance of myofibroblasts, generally indicated by α-SMA expression (Fig. 1) is used as a diagnostic tool to detect and grade the progress of fibro-contractive diseases.

2.2. Universal myofibroblast features

There is more to α-SMA than just being a myofibroblast marker. After formation of actin-myosin contractile bundles, stress fibers, it is the neo-expression and incorporation of α-SMA that significantly augments the contractile activity of activated myofibroblasts (Hinz et al., 2001). Similarly, induction of α-SMA in corneal stromal cells upregulates their contractile activity (Chen et al., 2007). Cell delivery of a peptide consisting the α-SMA-specific N-terminus causes the selective disassembly of α-SMA from stress fibers, associated with acute reduction of cell contraction and eventually decreased protein expression of collagen and α-SMA (Chaponnier et al., 1995; Clement et al., 2005; Hinz et al., 2002). In a recent study, our lab could show that expression of α-SMA alone is sufficient to direct the fate of mesenchymal stromal cells (MSC) by generating high intracellular stress (Talele et al., 2015).

There is also more to the activated myofibroblast than just expressing α-SMA. 'Activation' as a term is poorly defined and has been used to describe enhanced or newly acquired cell contraction, migration, proliferation, cytokine production, ECM secretion, and ECM degradation. From a pure metabolic point of view, all these functions are unlikely performed simultaneously in one single cell but rather characterize different stages in the lifetime of a myofibroblast or different types of myofibroblasts. The minimum requirements to define a myofibroblast are high contractile activity and the associated formation of stress fiber-like microfilament bundles *in vivo* (Hinz, 2010b). Although activated cells need to migrate to sites of injury and proliferate, both features are typically not used to characterize myofibroblasts. Intuitively, it is difficult to migrate and divide if the cytoplasm is filled with huge contractile bundles (Rønnov-Jessen and Petersen, 1996). The term 'proto-myofibroblast' has been proposed to discriminate early activated and more migratory fibroblasts from their quiescent tissue precursors that are devoid of a contractile apparatus (Tomasek et al., 2002). Ultimately, 'proto-myofibroblast' describes a stepping stone on the journey to become the mature α-SMA-expressing myofibroblast (Fig. 1).

Not every cell that expresses α-SMA is a myofibroblast. Not surprisingly given the actin isoform's name, smooth muscle cells (SMCs) express α-SMA, as do pericytes and myoepithelial cells (Arnoldi et al., 2012). However, these cells typically do not organize

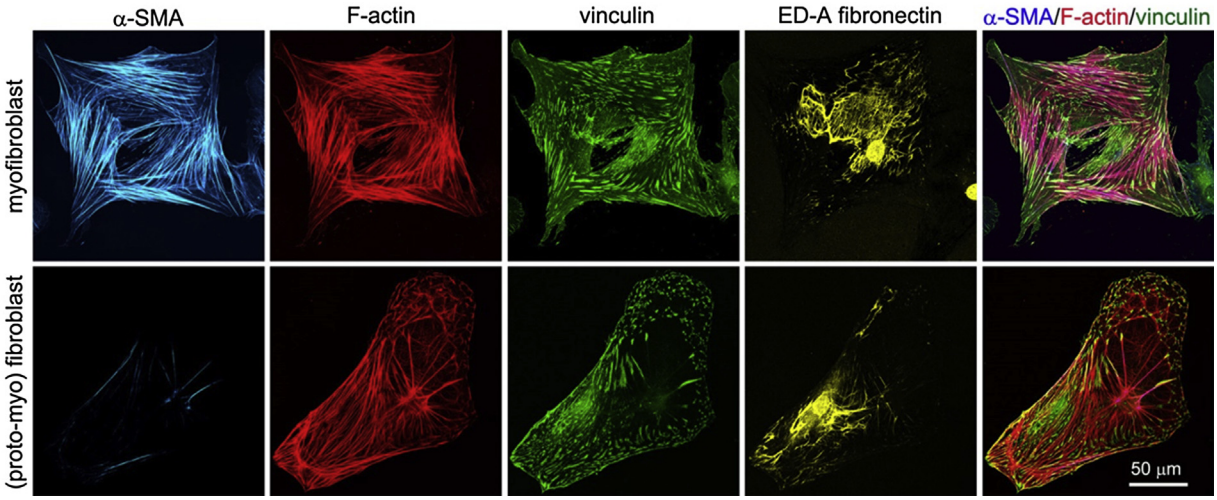

Fig. 1. *Myofibroblasts in vitro.* Primary fibroblasts were cultured for 4 days on conventional culture plastic dishes in the absence (bottom) or presence of TGF-β1 to induce myofibroblast activation (top). Cells were then stained for α-SMA (blue), F-actin-rich stress fibers (Phalloidin-red), vinculin (green), ED-A fibronectin and nuclei (both yellow).