Formation and Function of the Myofibroblast during Tissue Repair

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It is generally accepted that fibroblast-to-myofibroblast differentiation represents a key event during wound healing and tissue repair. The high contractile force generated by myofibroblasts is beneficial for physiological tissue remodeling but detrimental for tissue function when it becomes excessive such as in hypertrophic scars, in virtually all fibrotic diseases and during stroma reaction to tumors. Specific molecular features as well as factors that control myofibroblast differentiation are potential targets to counteract its development, function, and survival. Such targets include α-smooth muscle actin and more recently discovered markers of the myofibroblast cytoskeleton, membrane surface proteins, and the extracellular matrix. Moreover, intervening with myofibroblast stress perception and transmission offers novel strategies to reduce tissue contracture; stress release leads to the instant loss of contraction and promotes apoptosis.

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Introduction

It is generally accepted that myofibroblasts represent key players in the physiological reconstruction of connective tissue after injury and in generating the pathological tissue deformations that characterize fibrosis (Gabbiani, 2003; Desmouliere *et al.*, 2005). In previous reviews, we have focused on the role of cell–cell and cell–matrix contacts in myofibroblast development and function (Hinz and Gabbiani, 2003a, b; Hinz, 2006). Here, I draw a more general picture how the mechanical and chemical microenvironments integrate to promote tissue remodeling by myofibroblast. Differentiation of fibroblasts into myofibroblasts can be understood as a two-step process: (1) to re-populate damaged tissues, fibroblasts acquire a migratory phenotype by *de novo* developing contractile bundles. These *in vivo* stress fibers are first composed of cytoplasmic actins and generate comparably small traction forces (Hinz *et al.*, 2001b). We have recently proposed the term ''proto-myofibroblast'' to discriminate such activated fibroblasts from quiescent fibroblasts that are devoid of any contractile apparatus in most intact tissues (Tomasek *et al.*, 2002). This first phenotypic change occurs in response to changes in the composition, organization, and mechanical property of the extracellular matrix (ECM) (Hinz and Gabbiani, 2003b) and to cytokines locally released by inflammatory and resident cells (Werner and Grose, 2003). (2) With increasing stress in the ECM resulting from their own remodeling activity, proto-myofibroblasts further develop into ''differentiated myofibroblasts'' by neo-expressing α-smooth muscle actin (α-SMA), the most widely used myofibroblast marker. Expression of α-SMA is precisely controlled by the joint action of growth factors like transforming growth factor (TGFβ1), of specialized ECM proteins like the fibronectin (FN) splice variant ED-A FN, and of the mechanical microenvironment (Tomasek *et al.*, 2002). Incorporation of α-SMA into stress fibers significantly augments the contractile activity of fibroblastic cells and hallmarks the contraction phase of connective tissue remodeling (Hinz *et al.*, 2001a).

It has to be noted that the contribution of myofibroblast contraction to physiological tissue remodeling has been questioned by a study reporting normal closure of pig full thickness wounds despite repeated excision of the central and peripheral granulation tissue (Gross *et al.*, 1995). However, rat wounds that were kept open for 10 days with a plastic frame and have then been released from the splint contract to ~50% of their initial size within 6 hours; this cannot be explained by enhanced proliferation of fibroblasts and keratinocytes at the wound edge. Hence, the porcine skin may be able to compensate for the experimentally induced chronic loss of granulation tissue by mechanisms that have not yet been elucidated. Such a compensatory mechanism appears to function in α-SMA-knockout mice that macroscopically exhibit almost normal wound closure (personal communication, J.J. Tomasek, University of Oklahoma Health

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Abbreviations: α-SMA, α-smooth muscle actin; ECM, extracellular matrix; FA, focal adhesion; Fizz, found in inflammatory zone; FN, fibronectin; LAP, latency-associated protein; LTBP-1, latent TGFβ1-binding protein; SMC, smooth muscle cell; TGF, transforming growth factor

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Sciences Center, Oklahoma City, OK). These mice appear to substitute for α-SMA by *de novo* expressing other muscle actin isoforms like γ-smooth muscle and α-skeletal actin, which also explains why blood pressure is surprisingly little affected by the lack of α-SMA in vascular smooth muscle cells (SMCs) (Schildmeyer *et al.*, 2000). In contrast to these chronic conditions, acute inhibition of myofibroblast contraction by intracellular delivery of the specific N-terminal sequence AcEEED of α-SMA significantly reduces wound contraction (Hinz *et al.*, 2002). It will be interesting to test whether conditional α-SMA-knockout mice or mice treated with α-SMA RNAi will exhibit similarly impaired wound closure.

In physiological remodeling such as during dermal wound healing, the contractile activity of myofibroblasts is terminated when the tissue is repaired; α-SMA expression then decreases and myofibroblasts disappear by apoptosis (Desmouliere *et al.*, 1995). In pathological wound healing, however, myofibroblast activity persists and leads to tissue deformation, which is particularly evident in hypertrophic scars developing after burn injury, in the fibrotic phase of scleroderma and in the palmar fibromatosis of Dupuytren's disease (Schurch *et al.*, 2006). Myofibroblast-generated contractures are also characteristic for fibrosis affecting vital organs such as the liver (Desmouliere *et al.*, 2003), heart (Virag and Murry, 2003; Brown *et al.*, 2005), lung (Phan, 2002; Thannickal *et al.*, 2004), and kidney (Lan, 2003). In addition, myofibroblast participation to the process called stroma reaction promotes cancer progression by creating a stimulating microenvironment for epithelial tumor cells (De Wever and Mareel, 2003; Desmouliere *et al.*, 2004). It becomes increasingly accepted that stroma cells represent important targets of anticancer treatments (Bissell and Radisky, 2001; Liotta and Kohn, 2001; Mueller and Fusenig, 2004). In the light of such severe consequences of myofibroblast appearance and dysfunction, the necessity of more profoundly understanding the molecular mechanisms of myofibroblast formation and function appears clear.

Myofibroblast origin

The classical view on dermal wound healing implies local recruitment of fibroblasts from the dermis of the intact adjacent skin to sites of inflammation (Desmouliere *et al.*, 2005). Pericytes and SMC from the vasculature have been proposed as another local myofibroblast source in scleroderma (Rajkumar *et al.*, 2005) as well as liver and glomerular fibrosis (Desmouliere *et al.*, 2003). In addition, fibroblasts may originate from fibrocytes, a sub-population of bone marrow-derived leukocytes with fibroblast characteristics (Abe *et al.*, 2001). This concept has recently been revisited by transplanting bone marrow from (1) male to irradiated female mice and identifying the Y chromosome by *in situ* hybridization (Direkze *et al.*, 2003) and (2) from transgenic green fluorescent mice to irradiated wild-type animals (Hashimoto *et al.*, 2004). These studies reveal a surprisingly high fraction of 30–50% of the wound myofibroblasts potentially deriving from fibrocyte progenitors (Direkze *et al.*, 2003; Ishii *et al.*, 2005; Mori *et al.*, 2005). Comparable proportions have been demonstrated for myofibroblasts appearing during fibrosis of the liver (Forbes *et al.*, 2004), kidney (Direkze *et al.*, 2003), and lung (Hashimoto *et al.*, 2004) and during the stroma reaction to epithelial tumors (Ishii *et al.*, 2003). It remains to be seen whether myofibroblasts from different origins exhibit different characteristics and functions during tissue repair as suggested for liver fibrosis (Guyot *et al.*, 2006) or whether the organism is recruiting myofibroblast precursors from several sources to satisfy the temporarily high demand of contractile cells, which may all follow a similar differentiation program.

Identification of the myofibroblast – a warrant

The question of the myofibroblast origin is closely related to the problem of its identification. Three major ultrastructural features discriminate myofibroblasts from quiescent fibroblasts in tissues: (1) bundles of contractile microfilaments, (2) extensive cell-to-matrix attachment sites, and (3) intercellular adherens and gap junctions (Eyden, 2005; Schurch *et al.*, 2006). However, this definition has its limits when myofibroblasts need to be discriminated from other contractile cell types like SMC, requiring specific molecular markers.

Cytoskeletal markers of the myofibroblast. The most frequently employed myofibroblast marker is α-SMA, which evidently fails to distinguish between myofibroblasts and SMC in situations that exhibit mixed populations. For example, remodeling of injured arteries is thought to be predominantly driven by SMC from the media but a contribution from adventitial fibroblasts has also been suggested (Sartore *et al.*, 2001; Zalewski *et al.*, 2002). Contractile SMC specifically express smooth muscle myosin heavy chain, h-caldesmon, and desmin; however, SMC loose these markers when acquiring a synthetic phenotype and after being placed in culture (Christen *et al.*, 2001). Until recently, smoothelin was suggested a late differentiation marker for SMC that is not expressed in myofibroblasts (van der Loop *et al.*, 1996). However, gene expression profiling supported by protein biochemistry revealed induction of smoothelin and other late SMC markers in TGFβ1-treated cultured lung fibroblasts (Chambers *et al.*, 2003). Very recently, the 4Ig isoform of the stress fiber protein palladin has been proposed as novel marker for myofibroblast differentiation (Rönty *et al.*, 2006), but Western blotting analysis with pan-palladin antibodies indicates expression of this isoform also in SMC (Mykkanen *et al.*, 2001). Hence, at present no cytoskeletal protein allows to reliably discriminate between myofibroblasts and SMC; however, recent advances in proteomics and gene array analysis may lead to the identification of such a unique marker of the myofibroblast, provided that it exists (Chambers *et al.*, 2003; Malmstrom *et al.*, 2004).

The myofibroblast surface. Of particular interest not only for the purpose of