Molecular regulation of myofibroblast formation

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Novel strategies are required to counteract tissue contractures characteristic for organ fibrosis, stroma reaction to tumors, host reaction to implants, and formation of hypertrophic scars as after burns. Key element for the development and progression of these pathologies is the excessive contractile force generated by α-smooth muscle actin (α-SMA)-expressing myofibroblasts. We can modulate myofibroblast tension using multiple techniques, including collagen gels of different stiffness, novel substrates with tunable compliance, stretchable culture membranes and cell shape restriction by microcontact-printing. On the molecular level, we specifically interfere with the contractile apparatus, matrix-adhesion, cell-adhesion and growth factor activation of myofibroblasts. In general, stress-release leads to de-differentiation and/or apoptosis of myofibroblasts. We propose therapeutically interfering with their stress-perception and -transmission apparatus as innovative strategy to eliminate fibrogenic cells.

α-SMA as target for anti-fibrosis therapy

Hinz B, Celetta G, Tomasek J J, Gabbiani G, Chaponnier C. Alpha-smooth muscle actin expression up-regulates fibroblast contractile activity. Mol Biol Cell 2001: 12: 2730–2741.

The results of this work indicate correlation between the level of α-SMA expression and fibroblast contraction. In addition, new insights are provided into the interdependence of TGFβ1 and ED-A FN in regulating myofibroblast contractile activity.

Hinz B, Gabbiani G, Chaponnier C. The NH2-terminal peptide of alpha-smooth muscle actin inhibits force generation by the myofibroblast *in vitro* and *in vivo*. J Cell Biol 2002: 157: 657–663.

To elucidate the role of the α-SMA-specific N-terminus, the authors constructed a fusion peptide (SMA-FP) including the α-SMA N-terminal sequence Ac-EEED and the Antennapedia third helix sequence (pAntp-Pro50) that allows cell penetration. They show that the SMA-FP localizes in stress fibres rapidly after its delivery; it then inhibits contractility of fibroblasts *in vitro* and granulation tissue contraction *in vivo*, thus supporting the assumption that α-SMA plays an important role in wound contraction.

Wang J, Zohar R, McCulloch C A. Multiple roles of alpha-smooth muscle actin in mechanotransduction. Exp Cell Res 2006: 312: 205–214.

In this review the authors nicely elucidate the multiple roles of α-SMA as mechanotransducer, as contraction enhancing protein as mechanosensitive protein whose expression depends on a critical level of stress.

How matrix stiffness and stress determine myofibroblast fate

Arora P D, Narani N, McCulloch C A. The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. Am J Pathol 1999: 154: 871–882.

This hallmark paper from the McCulloch group for the first time demonstrated the dependence of α-SMA expression on the mechanical properties of the extracellular matrix. Using collagen substrates of different mechanical strength, the authors show that the potent myofibroblast inducer TGFβ1 is inefficient to upregulate α-SMA expression in the absence of stress.

Hinz B, Mastrangelo D, Iselin C E, Chaponnier C, Gabbiani G. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol 2001: 159: 1009–1020.

Two different rat models have been employed to evaluating: (1) the role of mechanical load in myofibroblast modulation (α-SMA expression in particular) and (2) in granulation tissue contractility. In the first model, the contraction of full thickness wound granulation tissue was prevented by splinting with a plastic frame. In the second model, the wall of rat granuloma pouches was released from mechanical tension by evacuating the pouch exudate. In both models isometric contraction and expression of the myofibroblast markers F-actin, ED-A FN, α-SMA, TGFβ-RII and TGFβ1 were evaluated. The results indicate that mechanical tension is crucial for differentiation and maintenance of the myofibroblast phenotype. It is moreover shown that tissue contractility correlates with the expression of α-SMA.

Goffin J M, Pittet P, Csucs G, Lussi J W, Meister J J, Hinz B. Focal adhesion size controls tension-dependent

recruitment of alpha-smooth muscle actin to stress fibers. J Cell Biol 2006: 172: 259–268.

Recruitment of α-SMA to pre-existing stress fibres requires a critical tension that is only generated upon formation of giant, so-called supermature focal adhesions on sufficiently rigid substrates. This work further demonstrates that supermature focal adhesions exert significantly higher force per unit area compared with classical focal adhesions of α-SMA-negative fibroblasts. Hence, establishment of supermature focal adhesions is a central checkpoint in the mechanical feedback loop of intracellular contractile activity and extracellular tension by controlling α-SMA recruitment to stress fibres.

Hinz B, Dugina V, Ballestrem C, Wehrle-Haller B, Chaponnier C. Alpha smooth muscle actin is crucial for focal adhesion maturation in myofibroblasts. Mol Biol Cell 2003: 14: 2508–2519.

The expression level of α-SMA in stress fibres correlates with the degree of focal adhesion maturation and the strength of fibroblast adhesion. Inhibition of myofibroblast contractile activity by the SMA-FP leads to the disassembly of supermature focal adhesions and decreases cell adhesion. This study proposes a model where expression of the contractile protein α-SMA increases the intracellular mechanical stress on focal adhesions and thereby induces their supermaturation.

Engler A J, Sen S, Sweeney H L, Discher D E. Matrix elasticity directs stem cell lineage specification. Cell 2006: 126: 677–689.

Although not related to wound healing and myofibroblast biology this work elegantly shows how powerful the influence of matrix stiffness is in regulating cell fate. Mesenchymal stem cells grown on substrates that correspond in stiffness to bone, muscle and brain develop into the corresponding cell lineages. The lineage determining effect of the matrix can even override conflicting information from chemical growth factors.

Carlson M A, Longaker M T, Thompson J S. Wound splinting regulates granulation tissue survival. J Surg Res 2003: 110: 304–309.

Grinnell F, Zhu M, Carlson M A, Abrams J M. Release of mechanical tension triggers apoptosis of human fibroblasts in a model of regressing granulation tissue. Exp Cell Res 1999: 248: 608–619.

Whereas stress is one major factor inducing the myofibroblast, release from stress appears to be a potent signal to trigger myofibroblast disappearance by apoptosis. These two studies demonstrate upregulation of apoptotic figures in fibroblastic cells using a stress-released collagen gel model (Grinnell) and stress released full thickness wounds (Carlson). It is assumed that *in vivo*, e.g. during wound healing, stress release occurs when the matrix is sufficiently remodelled to take over the mechanical load that occurs during tissue repair. Under these circumstances the architecture of the extracellular matrix stress shields its resident cells.

Where mechanical and chemical signals converge

Wells R G, Discher D E. Matrix elasticity, cytoskeletal tension, and TGF-β: the insoluble and soluble meet. Sci Signal 2008: 1: pe13.

This perspectives article nicely summarizes how the mechanical microenvironment influences physiological and pathological behaviour of different cell types in the context of wound healing and fibrosis. It comments on the articles cited below and establishes links between the scientific communities of mechanobiology, cell biology and clinical research.

Wipff P-J, Rifkin J P, Meister J-J, Hinz B. Myofibroblast contraction activates TGFβ1 from the ECM. J Cell Biol 2007: 179: 1311–1323.

This work on myofibroblast differentiation provides the first evidence that mechanical stress can lead to the direct activation of a growth factor. Both external stretching of myofibroblast cultures and increasing myofibroblast intracellular tension directly activate latent TGFβ1 from the ECM. This process requires α-SMA-positive stress fibres and integrin binding to the latent TGF-β1 complex. Latent TGF-β1 stress-activation is limited to culture substrates with stiffness similar to that of fibroblast-populated early wound granulation tissue but does not occur on more compliant substrates. We propose that ECM stiffness, modulated as a function of cell remodelling activity, controls the level of TGF-β1 release by contraction, and thus restricts autocrine maintenance of the myofibroblast phenotype to the appropriate mechanical microenvironment. This work builds up on the findings of Annes and co-workers and of Munger et al., listed below.

Annes J P, Chen Y, Munger J S, Rifkin D B. Integrin αvβ6-mediated activation of latent TGF-β requires the latent TGF-β binding protein-1. J Cell Biol 2004: 165: 723–734.

In this hallmark paper the authors demonstrate that activation of latent TGFβ1 by the epithelial integrin αvβ6 requires binding of the latent TGFβ1 complex to the extracellular matrix. It later turned out (see above) that the function of this binding may be to provide a mechanically resistant anchor point against the pulling of the integrin. However, this mechanism has not yet been proven for this particular integrin.

Munger J S, Huang X, Kawakatsu H, Griffiths M J, Dalton S L, Wu J, Pittet J F, Kaminski N, Garat C, Matthay M A, Rifkin D B, Sheppard D. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mecha-

nism for regulating pulmonary inflammation and fibrosis. Cell 1999: 96: 319–328.

This is one work out of a series of publications from the Sheppard lab showing that integrins are involved in the activation of latent TGFβ1. The reader is also referred to more recent publications of this group.

General reviews on the myofibroblast

Tomasek J J, Gabbiani G, Chaponnier C, Hinz B, Brown R A. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 2002: 3: 349–363.

During the past 30 years, it has become generally accepted that the modulation of fibroblastic cells towards the myofibroblastic phenotype, with acquisition of specialized contractile features, is essential for connective-tissue remodelling during normal and pathological wound healing. Yet the myofibroblast still remains one of the most enigmatic of cells, not least owing to its transient appearance in association with connective-tissue injury and to the difficulties in establishing its role in the production of tissue contracture. It is clear that our understanding of the myofibroblast – its origins, functions and molecular regulation – will have a profound influence on the future effectiveness not only of tissue engineering but also of regenerative medicine generally.

Hinz B. Formation and function of the myofibroblast. J Invest Dermatol 2007: 127: 526–537.

The high contractile force generated by myofibroblasts is beneficial for physiological tissue remodelling 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-SMA 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.

Hinz B, Phan S, Thannickal V, Galli A, Bochaton-Piallat M-L, Gabbiani G. The myofibroblast: one function, multiple origins. Am J Pathol 2007: 170: 1807–1816.

The crucial role played by the myofibroblast in wound healing and pathological organ remodelling is well established; the general mechanisms of extracellular matrix synthesis and of tension production by this cell have been amply clarified. This review discusses the pattern of myofibroblast accumulation and fibrosis evolution during lung and liver fibrosis as well as during atheromatous plaque formation. Special attention is paid to the specific features characterizing each of these processes, including the spectrum of different myofibroblast precursors and the distinct pathways involved in the formation of differentiated myofibroblasts in each lesion.

