

## TGF- $\beta$ gets primed for action

Study reveals that extracellular matrix remodeling by myofibroblasts enhances the activation of TGF- $\beta$ .

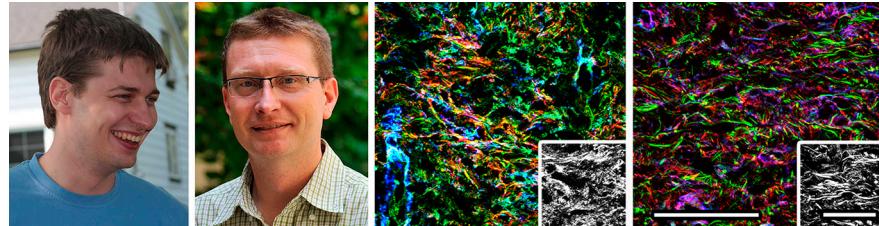
### FOCAL POINT

**M**yofibroblasts are highly contractile cells that repair damaged tissues by secreting and remodeling the extracellular matrix (ECM) within a wound in order to draw it closed (1). When myofibroblasts are dysregulated, however, they continue to act on healed tissues and induce fibrosis. TGF- $\beta$ 1 is a key signaling molecule within the ECM that promotes tissue fibrosis. Klingberg et al. reveal that the ECM-remodeling activity of myofibroblasts puts mechanical strain on latent TGF- $\beta$ 1 molecules, thereby priming them for activation (2).

TGF- $\beta$ 1 is secreted in an inactive form bound to latency-associated propeptide (LAP). This complex then binds to latent TGF- $\beta$ 1 binding protein (LTBP-1), which is incorporated into the ECM with other matrix proteins such as fibronectin (3). Boris Hinz and colleagues previously demonstrated that myofibroblasts activate latent TGF- $\beta$ 1 by pulling on LAP through their integrin adhesion receptors (4), inducing a conformational change that releases TGF- $\beta$ 1 (5).

"A more contractile cell pulls harder, making this process more efficient," explains Hinz, who now works at the University of Toronto. "But, if the TGF- $\beta$ 1 complex is linked to a floppy matrix, the mechanism won't work. You need a mechanically resistant matrix to create strain on LAP protein and open up the complex. So we hypothesized that whatever the myofibroblasts do to strain and stiffen the matrix would have an effect on TGF- $\beta$ 1 activation."

Hinz and colleagues, led by graduate student Franco Klingberg, first investigated how myofibroblasts influence ECM organization during wound healing in rats (2). The researchers found that wounds containing increased numbers of myofibroblasts exhibited much higher levels of ECM organization, with fibronectin and LTBP-1 arranged into extensive fibrils. The same was true in vitro: ECM produced by cultured human myofibroblasts was more



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**Franco Klingberg (left), Boris Hinz (right), and colleagues (not pictured) reveal that latent TGF- $\beta$ 1 complexes become primed for activation when myofibroblasts remodel the extracellular matrix into extended fibrils during wound healing. TGF- $\beta$ 1 complexes incorporated into these fibrils come under strain and are therefore more susceptible to being pulled apart and activated by myofibroblast-mediated contractility, an event that can lead to tissue fibrosis. Compared with a control wound (left) in which myofibroblasts are labeled for  $\alpha$ -smooth actin (blue), a nine-day-old dermal wound containing increased numbers of myofibroblasts (right) shows enhanced fibrillar organization of fibronectin (red) and latent TGF- $\beta$ 1 binding protein (green), resulting in higher levels of TGF- $\beta$ 1 activation.**

organized than matrix assembled by regular fibroblasts. In both cases, increased ECM organization correlated with enhanced TGF- $\beta$ 1 activation.

To directly test the contribution of ECM organization to TGF- $\beta$ 1 activation, Klingberg et al. compared the matrices assembled by wild-type and FAK-deficient mouse embryonic fibroblasts (MEFs). The mutant cells produced a highly disorganized ECM that nevertheless contained a similar amount

of latent TGF- $\beta$ 1 as the matrix produced by wild-type MEFs. Once the ECM was assembled, the researchers removed the MEFs from the matrices and replaced them with human myofibroblasts, which were able to release more than twice

as much active TGF- $\beta$ 1 from the organized matrix produced by wild-type MEFs.

To check that the disorganized matrix produced by FAK-null MEFs didn't have an inhibitory effect on myofibroblast contractility, Klingberg et al. coupled magnetic beads to anti-LAP antibodies that bind to the latent TGF- $\beta$ 1 complex. "If we pull on these beads, we can mechanically open the complex and release TGF- $\beta$ 1 without the involvement of any cells," Hinz says. "Using this approach, we were able to release more TGF- $\beta$ 1 from the organized matrix than from the floppy, disorganized matrix produced by FAK-null MEFs."

Klingberg et al. reasoned that the organization of LTBP-1 and latent TGF- $\beta$ 1 into fibrils would place the complex under strain, making it easier for myofibroblasts to pull the complex apart and release active growth factor. To test this idea, the researchers let normal fibroblasts secrete ECM onto silicone membranes that were subsequently stretched to straighten and strain the LTBP-1-containing fibrils to different extents. Contractile myofibroblasts were able to release more active TGF- $\beta$ 1 from matrices placed under greater levels of prestrain. "So we envision the matrix as a mechanical spring," Hinz explains. "As cells pull on it over time, the spring becomes completely extended. At that point, it will be very easy for the cells to induce a conformational change in the latent TGF- $\beta$ 1 complexes."

Because TGF- $\beta$ 1 induces further myofibroblast differentiation and, ultimately, tissue fibrosis, limiting ECM remodeling during wound healing might be an effective therapeutic approach. For now, though, Hinz and colleagues are interested in the interactions between myofibroblasts and macrophages, a major source of TGF- $\beta$ 1 in damaged tissues.

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