MYOFIBROBLASTS ORGANIZE THE LATENT TGF-β1 BINDING PROTEIN INTO PRE-STRESSED FIBRILLAR STRUCTURES: A POTENTIAL MECHANISM FOR EFFICIENT TGF-β1 RELEASE

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AIM OF THE STUDY

To test how the fibrillar organization state of the latent TGF-β1 binding protein (LTBP-1) affects TGF-β1 activation by myofibroblasts.

BACKGROUND

Myofibroblasts, generate the detrimental tissue contractures characteristic for fibrosis. Activation of myofibroblasts depends on two main factors: active TGF-β1 and mechanical stress.

Latent TGF-β1 is deposited in the extracellular matrix, (ECM) in complex with the storage protein LTBP-1 (Panel 1). Our previous research revealed a mechanical mechanism to liberate active TGF-β1 from the latent complex, involving "pulling" through myofibroblast integrins. For this mechanism to work, the ECM has to be mechanically resistant.

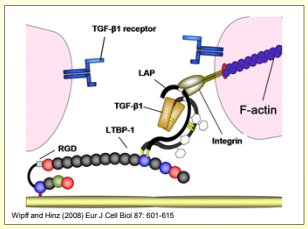
HYPOTHESIS

Mechanical priming of LTBP-1 by ongoing ECM remodelling will impact on the efficiency of TGF-β1 activation by myofibroblast contraction.

CONCLUSION

Mechanical priming of LTBP-1 fibrils during ECM maturation enhances TGF-β1 activation by human dermal myofibroblasts.

Panel 1: The mechanical release mechanism of active TGF-β1 from the latent TGF-β1 storage complex



- **TGF-β1** is secreted in a large latent complex, comprising the latency-associated peptide (LAP), and the latent TGF-β1 binding protein (LTBP-1). LAP and TGF-β1 intracellularly form the small latent complex.
- **LAP** contains an RGD recognition site for binding of different integrins. Upon integrin binding and force application, changes in LAP conformation result in release of active TGF-β1.
- TGF-β1 activation from the large latent complex by integrins requires binding of **LTBP-1** to the extracellular matrix (**ECM**). Binding is promoted at the LTBP-1 N-terminus that is transglutaminated to ECM proteins. LTBP-1 also contains an RGD sequence close to the ECM binding site, potentially acting as integrin ligand.
- It remains elusive whether **integrins** can directly bind to the RGD site in LTBP-1. We propose that cell pulling on LTBP-1 during fibril formation pre-stresses and thus mechanically primes the latent complex for efficient TGF-β1 activation by integrin pulling at the LAP moiety, analogous to loading a spring.

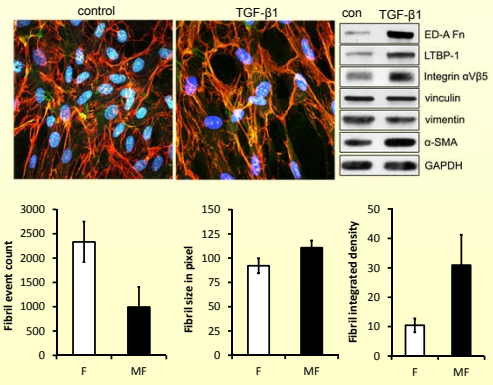
Panel 2 : Myofibroblasts differentiation changes ECM composition and structure

Rationale:

To quantify composition and structural differences in the ECM of fibroblasts and myofibroblasts.

Method:

Human dermal fibroblasts (HDFs) were grown without (control-con) and with TGF-β1 (+TGF-β1) for 7 days and assessed using WB and IF. Contraction of fibroblasts and myofibroblasts was induced and TGF-β1 release was measured using a reporter cell assay.



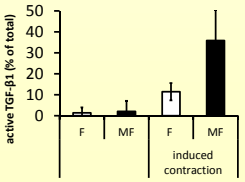
Panel 3: Myofibroblasts release more TGF-β1

Rationale:

Measure differences in TGF-β1 activation from the ECM.

Method:

Contraction of human dermal myofibroblasts (MF) and fibroblasts (F) was induced and TGF-β1 release was measured.



Panel 4: Increasing maturation of LTBP-1-containing ECM with culture time augments the levels of active TGF-β1

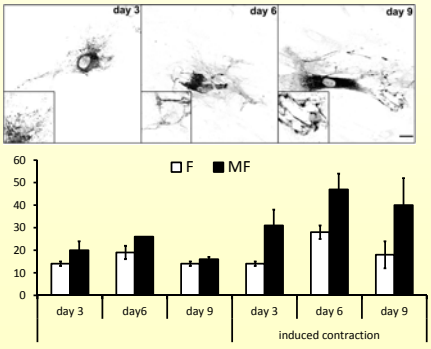
Rationale:

Test whether increasing LTBP-1 fibril formation over time has an effect on TGF-β1 activity.

Method:

(1) Visualization of fibril formation over time by transfecting LTBP-1-EGFP construct into HDFs.

(2) Measuring TGF-β1 activation by myofibroblasts (MF) and fibroblasts (F) from a pre-laid myofibroblast ECM.



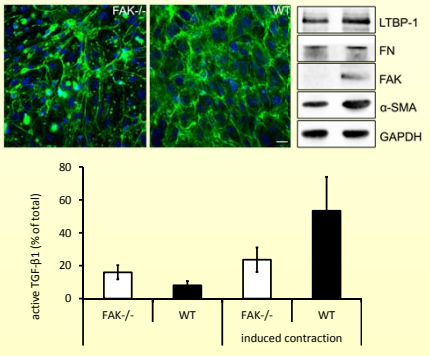
Panel 5: LTBP-1 fibrillar organization is essential for efficient TGF-β1 release

Rationale:

Test whether defective LTBP-1 fibril formation affects the ability of fibroblastic cells to activate TGF-β1.

Method:

Mouse embryonic fibroblasts (MEF) wild-type (WT) and focal adhesion kinase knock-out fibroblasts (FAK-/-) were grown for 7 days. MEFs were removed and myofibroblasts were seeded on top of the pre-laid ECM and induced to release TGF-β1 by contraction.



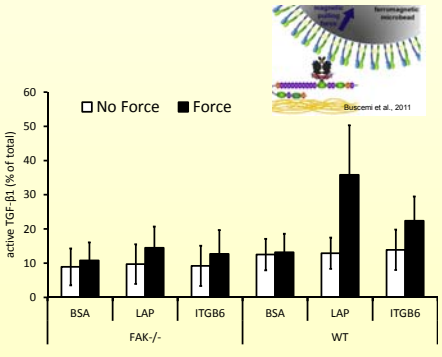
Panel 6 : Non-organized ECM impairs cell-free mechanical TGF-β1 release

Rationale:

To exclude biochemical effects of the mouse cell-produced ECM on HDF phenotype during TGF-β1 activation.

Method:

Ferromagnetic beads were coated with BSA, anti-LAP antibody or integrin αvβ6 and incubated with FAK-/- or WT ECM. Application of magnetic force resulted in the release of active TGF-β1.



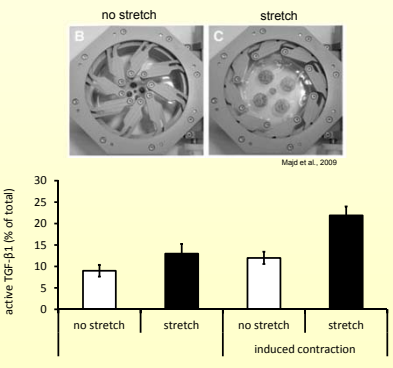
Panel 7: Mechanical pre-loading of LTBP-1 fibrils enhances TGF-β1 release

Rationale:

Test whether mechanical pre-loading of the LTBP-1 fibril containing ECM will facilitate TGF-β1 activation.

Method:

- HDFs were grown for 7 days on relaxed expandable silicone membranes (no stretch).
- Cells were then removed and the membrane was stretched to 190 % surface area (stretch).
- Myofibroblasts were seeded onto the pre-laid ECM in relaxed or stretched conditions and contraction was induced to release active TGF-β1.



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