Keeping fibroblasts in suspense: TAZ mediated signaling activates a context dependent pro-fibrotic phenotype

Bram Piersma¹, Ruud A. Bank¹*

¹ University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology, Matrix Research Group.

*corresponding author: Ruud A. Bank, r.a.bank@umcg.nl

Fibrosis in vital organs causes significant morbidity and mortality worldwide. Although our understanding of the cellular and molecular mechanisms underlying fibrosis has grown tremendously over the last decades, effective treatments that halt, reverse, or prevent the pathological accumulation and remodeling of extracellular matrix (ECM) are lacking. Recent advances revealed that ECM stiffening is one of the hallmarks of chronic fibrosis and stiff ECM is thought to be an active player in the development and progression of the disease (6). ECM stiffening can occur through a variety of processes initiated by the culprit of fibrosis: the myofibroblast. First, an imbalance between the production of ECM and activity of ECM-degrading enzymes shifts the balance toward ECM accumulation. Second, the sheer volume of collagens and other ECM components push out the interstitial fluids that keep tissues hydrated and compliant. Third, stretching of ECM by myofibroblasts renders the ECM less compliant in a process termed strain-stiffening. Finally, collagens and other ECM components become cross-linked via both enzymatic (LOX family; transglutaminases) and non-enzymatic mechanisms (2).

Myofibroblasts are connected with the surrounding ECM through specialized macromolecular assemblies (focal adhesions). Multiple signaling cascades have been implicated in the transduction of mechanical cues. The transcriptional co-activators from the Hippo pathway, YAP and TAZ, were found to act as mechanotransducers in epithelial cells. These findings were recently expanded to human fibrosis in the lung, liver, kidney, and palmar fascia, underlining the
clinical relevance of mechano-signaling cascades in ECM-related disorders (4, 5, 8, 9). Increased actin cytoskeletal tension due to increased ECM stiffening has been put forward as one of the mechanisms of YAP/TAZ activation (2, 10). Additionally, morphogens including TGFβ and WNT regulate YAP/TAZ activity and nuclear accumulation, adding to the complex regulation of these transcriptional modulators (7). How YAP and TAZ subsequently mediate the expression of pro-fibrotic genes remains incompletely understood.

In the current issue of the *American Journal of Physiology, Cell Physiology*, Jorgenson and coworkers elegantly describe how TAZ influences fibroblast activity and proliferation in both a scaffold-free spheroid system as well as a 2D culture system that employs polyacrylamide hydrogels in order to mimic the physiological stiffness of the ECM (6). When fibroblasts expressing a constitutively active mutant form of TAZ (replacement of four serine residues by alanine; TAZ4SA) were cultured in free-floating spheroids, they overcome the growth limitation posed by the ECM-free scaffold. The size of fibroblast spheroids was found to be increased compared to control cells as a result of cell proliferation. Additionally, they found that TAZ4SA increased the expression of the pro-fibrotic growth factors connective tissue growth factor (*Ctgf*), endothelin-1 (*Edn1*), and plasminogen activator inhibitor-1 (*Serpine1*). However, TAZ4SA did not affect the expression of collagens and fibronectin. The authors hypothesized that the inability to induce expression of ECM-related genes was due to the compliant nature of the spheroid cultures, which ranged from 0.5-3.5 kPa, similar to healthy lung tissues. Indeed, when the fibroblasts were instead grown on 2D hydrogels imitating pathological stiffness (75 kPa), TAZ4SA expression was sufficient to increase the expression of *Col1a1, Col1a2, Col1a3* and *Fn1* transcripts. This approach demonstrates that active TAZ requires the input of mechanical stimuli in order to facilitate expression of certain genes. Addition of the pro-fibrotic growth factor TGFβ1 further increased expression of this set of pro-fibrotic genes, whereas addition of the MRTF agonist ISX-9 did not. However, inhibition of MRTF did reduce TAZ4SA-induced *Col1a1* expression, suggesting MRTF/TAZ cross-talk on stiff matrices. What is intriguing, is that the genes encoding soluble morphogens do not require mechanical input, suggesting that the fibrotic response commences through growth factor expression, which drives myofibroblast activation and contraction of the ECM. Subsequent mechanosignaling through TAZ may act in concert with growth factor signaling to perpetuate the fibrotic response.
Previous findings from the authors described activation and propagation of the fibrotic response after orthotopic transplantation of TAZS4A fibroblasts into the lungs, raising the question as to how TAZS4A promotes ECM production in the highly compliant pulmonary tissue (4). As possible explanation, Jorgenson et al. postulated that TAZS4A increased the cells contractile activity, thereby strain-stiffening the immediate surrounding ECM and promoting mechanosensitive signaling. Indeed, they found TAZS4A fibroblasts to generate increased traction forces compared to control, even when grown on highly compliant substrates, providing data to support this hypothesis.

These findings continue to improve our understanding of the complex regulation of fibroblast activation by mechanical signaling, and shed additional light on the interplay between TAZ activation and TGFβ and MRTF signaling. Moreover, these results suggest that the transcriptional actions of TAZ depend on the mechanical context and possibly on the interaction with other mechanosensitive pathways, e.g. Rho/Rock and YAP. The fact that mechanical cues are intertwined with biochemical signals make it difficult to isolate specific signaling entities when studying fibroblast biology. The use of ECM-free spheroid cultures by Jorgensen et al. provides a straightforward and clean model for the study of fibrogenesis in the absence of mechanical input from the ECM. Although the data support a role for TAZ activation in fibrogenesis, several questions remain unanswered. First, how and why is TAZ activated in the initial stages of fibrosis development? Second, how exactly does TAZS4A activate fibroblasts proliferation, and can we translate this to myofibroblast proliferation in human lung pathologies? Third, what is the state of the cytoskeletal machinery in spheroid fibroblasts cultures? Finally, how does the exposure to growth factors such as TGFβ1 influence the cytoskeletal architecture in these cultures, and is this enough for TAZ4SA induced ECM production? Taken together, Jorgenson et al. have refined the understanding of TAZ activity and ECM production in fibroblasts by taken advantage of a 3D spheroid culture that mimics the compliance of healthy lung tissue.
References


Figure 1. Proposed actions of constitutively active TAZ (TAZ4SA) in context of ECM composition and stiffness. (A) Constitutively active TAZ in soft (0.5–3.5 kPa) 3D ECM-free spheroid cultures of mouse fibroblasts increases expression of growth factors but not ECM genes. (B) Constitutively active TAZ on soft (1 kPa) 2D collagen type I-functionalized substrates increases expression of Serpine1 and cellular contraction, but decreases expression of ECM genes. (C) Constitutively active TAZ on stiff (75 kPa) 2D collagen type I-functionalized substrates increases expression of Serpine1 and ECM genes, as well as cellular contraction. ↑, increased expression; ↓, decreased expression; ↔, no change in expression. kPa, Kilo Pascal; TAZ, Transcriptional coactivator with PDZ-binding motif.
A. ECM-free 3D spheroid (soft)

- ↑ proliferation
- ↑ Ctgf
- ↑ Edn1
- ↑ Serpine1
- ↔ ECM genes

B. Collagen-I coated 2D substrate (soft)

- ↑ Serpine1
- ↓ Col1a1
- ↓ Col1a2
- ↓ Col3a1
- ↔ Fn1
- ↑ cellular contraction

C. Collagen-I coated 2D substrate (stiff)

- ↑ Serpine1
- ↑ Col1a1
- ↑ Col1a2
- ↑ Col3a1
- ↑ Fn1
- ↑ cellular contraction