EDITORIAL FOCUS

Keeping fibroblasts in suspense: TAZ-mediated signaling activates a context-dependent profibrotic phenotype. Focus on “TAZ activation drives fibroblast spheroid growth, expression of profibrotic paracrine signals, and context-dependent ECM gene expression”

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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 nonenzymatic mechanisms (1).

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 coactivators from the Hippo pathway, YAP and TAZ (transcriptional coactivator with PDZ-binding motif), 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 mechanosignaling 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 TGF-β 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 profibrotic genes remains incompletely understood.

In the current issue of the American Journal of Physiology-Cell Physiology, Jorgenson and coworkers (3) 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 to mimic the physiological stiffness of the ECM. When fibroblasts expressing a constitutively active mutant form of TAZ (replacement of four serine residues by alanine; TAZS4A) were cultured in free-floating spheroids, they overcame the growth limitation posed by the ECM-free scaffold. The size of fibroblast spheroids was found to be increased compared with control cells as a result of cell proliferation. Additionally, they found that TAZS4A increased the expression of the profibrotic growth factors connective tissue growth factor (Ctgf), endothelin-1 (Edn1), and plasminogen activator inhibitor-1 (Serpine1). However, TAZS4A 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 to 3.5 kPa, similar to healthy lung tissues. Indeed, when the fibroblasts were instead grown on 2D hydrogels imitating pathological stiffness (75 kPa), TAZS4A expression was sufficient to increase the expression of Coll1a1, Coll1a2, Colla3, and Fn1 transcripts. This approach demonstrates that active TAZ requires the input of mechanical stimuli to facilitate expression of certain genes (Fig. 1). Addition of the profibrotic growth factor TGF-β1 further increased expression of this set of profibrotic genes, whereas addition of the myocardin-related transcription factor (MRTF) agonist ISX-9 did not. However, inhibition of MRTF did reduce TAZS4A-induced Coll1a1 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 (Fig. 1).

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 TAZ4SA fibroblasts to generate increased traction forces compared with 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 TGF-β/Hippo 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 makes it difficult to isolate specific signaling entities when studying fibroblast biology. The use of ECM-free spheroid cultures by Jorgenson et al. (3) provide 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 TAZ4SA 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 TGF-β1 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 taking advantage of a 3D spheroid culture that mimics the compliance of healthy lung tissue.

**AUTHOR CONTRIBUTIONS**

B.P. and R.A.B. drafted manuscript; B.P. and R.A.B. edited and revised manuscript; B.P. and R.A.B. approved final version of manuscript.

**DISCLOSURES**

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**REFERENCES**


