FIBROSIS

PU.1 pulls the strings in fibrotic disease

> PU.1 has a central role in fibroblast fate and function

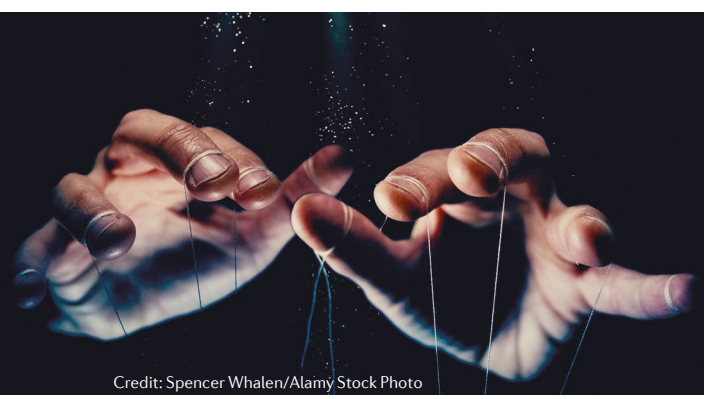
According to new research published in *Nature*, the ETS family transcription factor PU.1 can function as a switch controlling the polarization of fibroblasts and thereby the contribution of these cells to disease.

Fibroblasts can be divided into two types of potentially pathogenic cells. Extracellular matrix (ECM)-producing 'fibrotic fibroblasts' are overactive in fibrotic diseases such as systemic sclerosis (SSc), whereas ECM-degrading 'inflammatory fibroblasts' occur more readily in chronic inflammatory diseases, such as rheumatoid arthritis (RA).

The researchers noticed a possible contribution of PU.1 to fibrosis during a bioinformatic screen of promoter regions of profibrotic genes taken from a database of skin samples from patients with SSc. PU.1 was the most enriched transcription factor in this analysis, indicating a major regulatory function.

PU.1 is already a well-characterized transcription factor known to have a central function in the development of B cells and myeloid cells, but little is known of its effect on fibroblasts, fibrosis and extracellular remodelling, until now.

The new data show that PU.1 is highly expressed by fibroblasts in fibrotic skin, liver, lung and kidney biopsy samples from patients with SSc. By contrast, PU.1 was not expressed by fibroblasts from non-fibrotic healthy or inflamed tissues.



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The researchers demonstrated the functionality of PU.1 in fibrosis by comparing the effect of PU.1 overexpression versus knockout on human fibroblasts. After knockout of *SPI1* (the gene encoding PU.1) using CRISPR–Cas9, fibroblasts from fibrotic tissues lost the characteristic expression of α-smooth muscle actin and produced less collagen. The reverse occurred in resting fibroblasts made to transgenically overexpress *SPI1*. Overexpression of *SPI1* was also sufficient to convert inflammatory fibroblasts into fibrotic fibroblasts. In organoid cultures designed to mimic the synovial membrane, *SPI1* transgenic fibroblasts were unable to form lining layers, and in full-thickness skin organoid cultures they started producing ECM and a thickened dermal layer.

The major finding of high expression of PU.1 specifically in fibrotic fibroblasts was replicated in mouse models of bleomycin-induced skin and lung fibrosis, and in CCL_4-induced liver fibrosis. Furthermore, disease was prevented in these mice by fibroblast-specific deletion of *Spi1*.

But how is PU.1 activated and how does it confer this fibrotic phenotype on fibroblasts?

The authors posit epigenetic mechanisms to account for differential PU.1 activity in the different fibroblast subpopulations, including a possible function of histone methylation marks in the upstream regulatory element and promoter of *SPI1*, as well as a role for microRNAs, including miR-155. miR-155, which is associated with various inflammatory diseases, seems to inhibit PU.1 expression and indeed the researchers show that mir-155 is highly expressed specifically by inflammatory fibroblasts and not by fibrotic fibroblasts.

As for how PU.1 in turn can control fibroblast fate and function, the researchers used chromatin immunoprecipitation (ChIP) to conclude, in line with the results from their initial bioinformatic screens, that PU.1 binds to important profibrotic genes, including *ACTA2* and *COL1A1*.

"PU.1 should not be considered as one major transcription factor that leads to aberrant fibrotic behaviour by its upregulation alone," explains corresponding author Andreas Ramming. Indeed, using PU.1 ChIP sequencing his team identified a network of transcription factors that bind near PU.1 binding sites to drive the fibrotic phenotype. This panel of transcription factors is distinct from those that are known to function in concert with PU.1 in other cell types. Ramming is also keen to point out that although there is clearly a network of regulatory factors, PU.1 has a central role in fibroblast fate and function. "PU.1 inhibition interrupts the complex cellular machinery of factors that drive the differentiation towards a fibrotic phenotype," he says "Therefore, PU.1 should be considered as an anchor of this fibrotic phenotype and its pharmacological inactivation might restore tissue homeostasis in several fibrotic diseases."

As evidence of this therapeutic potential, the researchers show that fibrosis is almost entirely prevented in liver, lung and skin fibrosis mouse models by treating the mice with the PU.1 inhibitor DB1976.

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