FIBROTIC DISEASE

A pair of novel targets

Fibrosis — the development of excess connective tissue owing to activation of myofibroblasts — is a key process in the pathophysiology of conditions such as lung injury and kidney disease. Two recent papers in *PNAS* and *Nature Medicine* highlight potential new targets for these conditions, which are often not responsive to existing drugs.

In the first study, Hecker and colleagues identified *NOX4* — the gene that encodes NADPH oxidase 4 (NOX4), which catalyzes the reduction of O_2 to reactive oxygen species — as one of the most highly induced genes in human foetal lung mesenchymal cells that had been stimulated with transforming growth factor β1 (TGFβ1) to induce differentiation into myofibroblasts. Studies using RNA interference-mediated knockdown of *NOX4* in lung mesenchymal cells isolated from individuals with human idiopathic pulmonary fibrosis showed that NOX4 was necessary for TGFβ1-stimulated hydrogen peroxide production and the induction of α-smooth muscle actin (αSMA, a marker of myofibroblast formation) and fibronectin expression. In addition, secretion of soluble collagen by TGFβ1-stimulated cells, was inhibited by knockdown of *NOX4*, supporting the involvement of NOX4 in myofibroblast differentiation and proliferation.

Next, the authors studied mouse models of lung injury. NOX4 expression was induced in a time-dependent manner during the fibrogenic phase of bleomycin-induced lung injury. In this model, and in a hapten-driven lung injury model, delivery of *Nox4*-specific small interfering RNA mediated an antifibrotic effect. Finally, the flavoenzyme inhibitor diphenyleneiodonium chloride reduced fibrosis in mice that were subjected to bleomycin-induced lung injury, and reduced numbers of αSMA-expressing myofibroblasts in the injured lung.

The second study built on increasing evidence that Ca^{2+} channels are involved in cellular proliferation by enhancing intracellular Ca^{2+} signalling and affecting cell cycle progression. Grgic and colleagues investigated the activity of the intermediate-conductance Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ (also known as KCNN4) in the development of renal fibrosis — a condition that often leads to chronic kidney failure.

In murine renal fibroblasts, mitogenic stimulation by basic fibroblast growth factor (bFGF) upregulated $K_{Ca}3.1$ expression, which was mediated by receptor tyrosine kinase activity. Blockade of $K_{Ca}3.1$ with the selective inhibitor TRAM-34 reduced proliferation of renal fibroblasts that had been stimulated by bFGF, and caused cell cycle arrest in phase G_0–G_1.

In mice that had undergone unilateral ureteral obstruction (UUO) — a model of renal fibrosis — there was more than a 20-fold increase in $K_{Ca}3.1$ expression in the kidneys, which was accompanied by an increased expression of fibroblast-specific protein 1, collagen I and III and TGFβ. Kidneys from $K_{Ca}3.1$-deficient mice that were subjected to UUO had attenuated chronic tubulointerstitial damage, reduced collagen deposition, fewer αSMA-positive cells and a better preservation of differentiated proximal tubules and total renal parenchyma compared with wild-type mice, showing that progression of renal fibrosis is attenuated by an absence of $K_{Ca}3.1$ channel functions. Finally, in the wild-type UUO model, injections of TRAM-34 attenuated renal fibrosis, which was accompanied by a reduction of chronic tubulointerstitial damage, a decrease in collagen I and III deposition and a significant reduction in interstitial αSMA-expressing cells.

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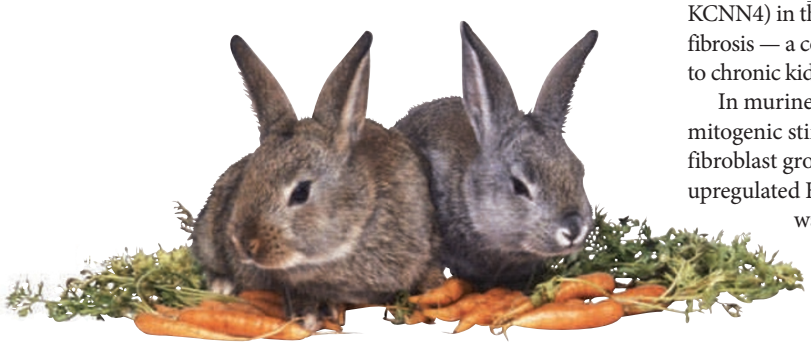

ORIGINAL RESEARCH PAPERS Hecker, L. *et al.* NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. *Nature Med.* **15**, 1077–1081 (2009) (doi:10.1038/nm.2005) | Grgic, I. *et al.* Renal fibrosis is attenuated by targeted disruption of $K_{Ca}3.1$ potassium channels. *Proc. Natl Acad. Sci. USA* **106**, 14518–14523 (2009)