NADPH oxidase DUOX1 sustains TGF-β1 signalling and promotes lung fibrosis

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ABSTRACT Interstitial lung fibroblast activation coupled with extracellular matrix production is a pathological signature of pulmonary fibrosis, and is governed by transforming growth factor (TGF)-β1/Smad signalling. TGF-β1 and oxidative stress cooperate to drive fibrosis. Cells can produce reactive oxygen species through activation and/or induction of NADPH oxidases, such as dual oxidase (DUOX1/2). Since DUOX enzymes, as extracellular hydrogen peroxide (H_2O_2)-generating systems, are involved in extracellular matrix formation and in wound healing in different experimental models, we hypothesised that DUOX-based NADPH oxidase plays a role in the pathophysiology of pulmonary fibrosis.

Our *in vivo* data (idiopathic pulmonary fibrosis patients and mouse models of lung fibrosis) showed that the NADPH oxidase DUOX1 is induced in response to lung injury. DUOX1-deficient mice ($DUOX1^{+/-}$ and $DUOX1^{-/-}$) had an attenuated fibrotic phenotype. In addition to being highly expressed at the epithelial surface of airways, DUOX1 appears to be well expressed in the fibroblastic foci of remodelled lungs. By using primary human and mouse lung fibroblasts, we showed that TGF-β1 upregulates DUOX1 and its maturation factor DUOXA1 and that DUOX1-derived H_2O_2 promoted the duration of TGF-β1-activated Smad3 phosphorylation by preventing phospho-Smad3 degradation. Analysis of the mechanism revealed that DUOX1 inhibited the interaction between phospho-Smad3 and the ubiquitin ligase NEDD4L, preventing NEDD4L-mediated ubiquitination of phospho-Smad3 and its targeting for degradation.

These findings highlight a role for DUOX1-derived H_2O_2 in a positive feedback that amplifies the signalling output of the TGF-β1 pathway and identify DUOX1 as a new therapeutic target in pulmonary fibrosis.