Understanding the Role of TRPV4 in Mechanosensing, Myofibroblast Differentiation and Pulmonary Fibrosis

By Mitchell A. Olman, MD

Idiopathic pulmonary fibrosis (IPF) is a fatal fibrotic lung disorder with marginally effective medical treatment. Myofibroblasts are critical to the fibrogenic lung repair process through their ability to produce collagen, secrete pro-fibrotic cytokines and contract tissue. Although myofibroblasts require active transforming growth factor-β (TGF-β), and a mechanical signal for their generation, the nature of the mechanical signal and the mechanism by which the mechanical signal is sensed have remained elusive.

In a search through the literature, my team noted that a calcium-permeable cell membrane ion channel named transient receptor potential vanilloid 4 (TRPV4) was activated upon plasma membrane stretch and thus could act as a mechanosensor. Thus, we investigated whether TRPV4 plays a role in myofibroblast differentiation and/or in vivo lung fibrosis.

In work recently published in the Journal of Clinical Investigation,1 we show that inhibition of TRPV4 by small molecule inhibitors and/or downregulation/deletion of TRPV4 using molecular or genetic techniques resulted in an almost complete blockade of both the calcium influx response as well as the myofibroblast differentiation response to TGF-β.

Moreover, TRPV4 exhibited its mechanosensing and myofibroblast-differentiating effect under conditions of matrix stiffness in the range of normal and fibrotic lung (1-25 kPa), suggesting it can sense changes in matrix stiffness during the fibrogenic process. Using a model assay system developed in our laboratory, we also noted that TRPV4 blocked the myofibroblast differentiation response to actual fibrotic lung tissue. Furthermore, TRPV4 deficiency protects mice from in vivo lung fibrosis in an experimental murine model.

These data identify TRPV4 as a long-elusive mechanosensor/transducer that mediates myofibroblast differentiation and in vivo pulmonary fibrogenesis. Successful manipulation of TRPV4 channel activity may be a novel therapeutic approach for fibrotic diseases of the lung and other organs. TRPV4 has also recently been shown to be involved in other disorders associated with pulmonary parenchymal stretch including ventilator-induced lung injury, and a TRPV4 inhibitor for pulmonary edema due to pulmonary venous hypertension is undergoing Phase I trials.

Successful manipulation of TRPV4 channel activity may be a novel therapeutic approach for fibrotic diseases of the lung and other organs.

Dr. Olman is a staff physician in the Respiratory Institute with a primary appointment in the Department of Pathobiology. Contact him at 216.445.7191 or olmanm@ccf.org.

Reference
Figure. TRPV4 is required for TGF-β1-induced lung myofibroblast differentiation.

HLFs were plated on fibronectin-coated (10 μg/mL plastic wells and incubated with or without TGF-β1 (2 ng/mL, 24 hours), TRPV4 siRNA or scrambled siRNA. (A) Representative immunoblots show knockdown of TRPV4 proteins by TRPV4-specific siRNA and blocking of TGF-β1–induced α-SMA expression under conditions of TRPV4 knockdown. (B and C) Quantification of (B) TRPV4/GAPDH and (C) α-SMA/GAPDH protein bands from A. *P < 0.05 scrambled vs. TRPV4 siRNA-treated cells, #P < 0.05 TGF-β1-treated cells treated with scrambled siRNA vs. TRPV4 siRNA; N = 3. (D) Representative fluorescence micrographs (original magnification, ×20). Myofibroblast differentiation is reduced in fibroblasts from TRPV4 KO mice (colocalization of α-SMA and F-actin, orange). (E) Quantification of results from D by Pearson's coefficient analysis. **P < 0.01; TGF-β1–treated WT vs. Trpv4 KO cells; N > 18 cells per group. UT, untreated. (F) Reconstitution of TRPV4 into Trpv4 KO mouse lung fibroblasts (MLFs) using a Lentivirus expression system (lenti-TRPV4-GFP) restores myofibroblast differentiation in response to TGF-β1. Lenti-GFP–infected Trpv4 KO mouse lung fibroblasts were used as negative controls; uninfected WT mouse lung fibroblasts were used as positive controls. Original magnification, ×20. (G) TRPV4 blockade has a greater inhibitory effect on myofibroblast differentiation (α-SMA/GAPDH band density in immunoblots) in fibroblasts from patients with IPF than in normal fibroblasts. (H) Quantitation of results from G. *P < 0.05; N = 5 per group. Results are expressed as mean ± SEM.

Citation: J Clin Invest. 2014;124(12):5225-5238. doi:10.1172/JCI75331.