A Focus on "Eye on" Channels in Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a lethal fibrotic lung disease with no cure (1). Fibroblasts are key effector cells in the pathogenesis of lung fibrosis (2). In response to soluble (e.g., TGF-β [transforming growth factor β]) and insoluble (e.g., extracellular matrix) signals, fibroblasts transdifferentiate into myofibroblasts to drive the fibrotic process (3–5). Other investigators and we have shown that myofibroblast differentiation is dependent on extracellular calcium influx (6). The mechanosensitive cation channel TRPV4 (transient receptor potential vanilloid 4) and voltage-gated L-type channels join a growing list of plasma membrane, cation-permeable channels that regulate calcium influx, myofibroblast transdifferentiation, and experimental pulmonary fibrosis (PF) *in vivo* (6, 7). Based on their differential expression in lungs of patients with idiopathic PF (IPF), as discovered on gene arrays, large-conductance, calcium-activated potassium channels, such as BK (big conductance potassium) channels, have recently garnered much interest (8). BK channel opening allows potassium efflux from the cell along its concentration gradient in response to changes in voltage or elevated intracellular calcium concentrations (9). BK channel activation plays a major role in vascular smooth muscle cell excitation–contraction coupling, resulting in smooth muscle cell relaxation leading to vasodilation (9). In addition, BK channels appear to play a secondary role in abrogating specific agonist-induced airway smooth muscle contraction and having effects on airway hydration, neural respiratory control, and cell volume regulation (9, 10). However, a role for BK channels in the activation and transdifferentiation of fibroblasts into myofibroblasts has not been described until now.

In this issue of the *Journal*, Scruggs and colleagues (pp. 191–203) report that fibroblasts from patients with IPF exhibit increased expression of the *KCNMB1* (potassium calcium-activated channel subfamily M regulatory β subunit 1) gene (11), which was previously found to be differentially methylated (8). *KCNMB1* is a gene that encodes for the β1 subunit of the calcium-activated BK channel family. The β subunits (β1–β4) *1*) pair with the calcium- and voltage-sensing, potassium pore–forming α subunit; *2*) regulate all key functions of the BK channel (e.g., pharmacologic properties, Ca^{2+}/voltage sensitivities, and kinetics); *3*) exhibit tissue-type– and isoform-specific expression; and *4*) confer the BK channel's pleiotropic actions (9). In addition, the BK channel's actions can be regulated by chemical ligands in a tissue-type–dependent manner, by its expression levels, and/or by its trafficking to the plasma membrane, and the channel can be sensitized by its phosphorylation (9, 12). Scruggs and colleagues demonstrate that the activity of BK channels is higher in fibroblasts from patients with IPF than in those from normal subjects, and the loss of BK function impairs fibroblast gel contraction in response to TGF-β. Furthermore, their studies in lung fibroblasts reveal that *1*) TGF-β induces *KCNMB1* expression, *2*) TGF-β induces a greater increase in intracellular calcium upon costimulation with a BK channel agonist, and *3*), confirming prior work, *ACTA2* (α-smooth muscle actin) expression is calcium dependent. Together, these data demonstrate that BK channels are an important mediator of TGF-β–induced myofibroblast transdifferentiation (Figure 1). Given their calcium-activation mechanism, the possibility that BK channels engage in cross-talk with other ion channels should be systematically and rigorously explored. As an example, cross-talk between the TRPV4 and BK channels has been shown to mediate cell volume recovery after hypotonic challenge (6, 9, 13).

Intracellular ion channels/pumps have emerged as key regulators of cellular functions, in part, by modulating local concentrations of ions (14). Calcium has been shown to act as a second messenger to mediate essential fibroblast functions, such as myofibroblast transdifferentiation (6, 7). However, the effects of calcium-activated potassium channels on fibroblast function are poorly understood. This study provides new insights into the pleiotropic actions of the BK channel on induction of myofibroblast transdifferentiation and contraction of pulmonary fibroblasts. Although the action of BK channels in fibroblasts is the opposite of that observed in smooth muscle cells, it mirrors their effect in other mesenchymal cells, such as synoviocytes and dermal fibroblasts (12).

The DNA methylated gene *KCNMB1*, which encodes for the BK channel studied in this work, was previously found to be a highly expressed gene in a survey of lung tissue from patients with IPF compared with normal lung tissue (8). There are many *KCNMB* genes (β1–β4) that encode for multiple BK channels. The *KCNMβ1* channel is highly expressed in lung fibroblasts as compared with its β2–β4 subunit expression. Furthermore, the authors show that expression of the β1 subunit is increased in IPF fibroblasts, and the β1 siRNA knockdown data demonstrate that β1 is necessary to induce myofibroblast transdifferentiation into lung fibroblasts.

The cell-type specificity of the BK channel β1 subunit may allow for targeted actions through therapeutic manipulation. In addition, there is the possibility that BK channels couple with other ion channels in a tissue- or disease-specific manner. For example, calcium channel blockers, calcineurin inhibitors, and TRPV4 channel deletion have been shown to abrogate bleomycin-induced experimental PF in mice (6, 7, 15). Furthermore, given the heterogeneity of *KCNMB1* expression in patients with IPF, this work may provide an avenue for personalized targeted therapy. Further studies of ion channels will likely provide better insight into the biology of myofibroblast transdifferentiation and fibrosis. Many unanswered questions remain to be addressed that can guide future research. For example, what is the mechanism of BK channel activation in response to TGF-β? If it is calcium, which calcium channels are involved and how are they activated? Precisely how

Supported by National Institutes of Health grants HL-133380 (R.G.S.) and HL-119792 and HL-133721 (M.A.O.).

Originally Published in Press as DOI: 10.1165/rcmb.2019-0343ED on October 17, 2019

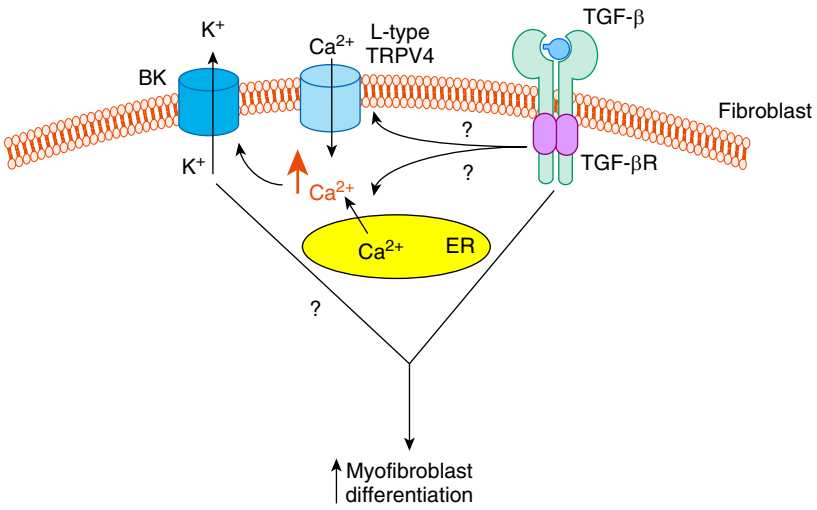

Figure 1. Proposed model for the cross-talk between cation channels, including the BK (big conductance potassium) channel and TGF-β (transforming growth factor β) signaling pathways in pulmonary fibrosis. This schematic is based on the findings of Scruggs and colleagues (11), which demonstrate that calcium-dependent potassium influx through BK channels cooperates with TGF-β to induce myofibroblast transdifferentiation via a calcium-dependent mechanism that remains to be determined. ER = endoplasmic reticulum; L-type= voltage activated calcium channel; TRPV4 = transient receptor potential vanilloid 4.

does activation of the BK channel alter the myofibroblast response to TGF-β—through ion flux or downstream signal mediators? Finally, what are the critical cell types and actions of BK channels that mediate PF *in vivo*? It is well known that calcium's signaling specificity can be encoded in its spatiotemporal variation patterns (7). Thus, a detailed examination of calcium fluxes using real-time analysis at the subcellular level of resolution would be warranted to begin to address some of these key unresolved questions.

In summary, the identification of novel plasma membrane channels that regulate myofibroblast transdifferentiation makes a significant contribution to the fibrosis field, and may provide a therapeutic target in IPF. In the study by Scruggs and colleagues, the BK channel was shown to affect myofibroblast transdifferentiation in a calcium-dependent manner. The BK channel now joins other cation/calcium channels, such as TRPV4 and L-type channels, as potential therapeutic targets to treat pulmonary (and potentially other) fibrotic disorders. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Rachel G. Scheraga, M.D.
Mitchell A. Olman, M.A., M.D.
Respiratory Institute
and
Lerner Research Institute
Cleveland Clinic Foundation
Cleveland, Ohio

ORCID IDs: 0000-0001-5113-543X (R.G.S.); 0000-0003-3262-3598 (M.A.O.).

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