TRP Channels in Pulmonary Fibrosis: Variety Is a Spice of Life

Pulmonary fibrosis is a devastating disease with available pharmacologic therapy that only slows progression and lacks curative potential (1). Fibroblasts differentiating into myofibroblasts play a vital role in the pathogenesis of lung fibrosis (2). Hence, specific myofibroblast targets to reverse or resolve fibrosis are desperately needed. Transient receptor potential (TRP) channels are a family of plasma membrane cation channels with unique functions in a variety of cell types (3). Seven TRP families have been discovered, which are categorized on the basis of sequence homology, including TRPV (vanilloid) and TRPA (ankyrin) (3, 4). We and others have shown that myofibroblast differentiation is dependent on cations (e.g., calcium) (5). Others have implicated calcium through non-TRP channels, such as L-type and T-type calcium channels, to mediate pulmonary fibrosis (6). Our group has discovered that the mechanosensitive cation channel TRPV4 (TRP vanilloid 4) is important in myofibroblast differentiation and experimental pulmonary fibrosis *in vivo* (5). In contrast to the mechanosensitive properties of TRPV4, TRPA1 is activated by extreme temperatures or compounds with spicy or pungent scents, including cinnamon, onion, garlic, and mustard (7). TRPA1 is well-known to play a role in the pathobiology of respiratory diseases, including cough and asthma (e.g., neurons and epithelial cells); however, its role in parenchymal lung disease is less certain (7, 8).

In this issue of the *Journal*, Geiger and colleagues (pp. 314–325) show that TRPA1 plays a role in profibrotic signaling in human lung fibroblasts (HLFs) (9). Specifically, an reverse transcriptase–polymerase chain reaction screen revealed that TRPA1 mRNA was downregulated by TGFβ in primary HLFs from normal subjects. Concordantly, TGF-β1 downregulated TRPA1 mRNA expression and channel function as measured by an intracellular calcium-sensitive dye. Basal downregulation of TRPA1 (with TRPA1 siRNA), increased mRNA expression of genes encoding several fibrosis markers (ACTA2 [α-smooth muscle actin], SERPINE1 [plasminogen activator inhibitor 1], *FN1* [fibronectin], and *COL1A1* [type I collagen]) in HLFs. Importantly, treatment of HLFs with a TRPA1 agonist (AITC and J7010) blocked the TGF-β induction of α smooth muscle actin and collagen I proteins. Collectively, these data suggest that TRPA1 downregulation by TGFβ results in a loss of TRPA1's inhibitory function on fibrotic gene expression. The findings, which address the mechanism by which TRPA1 exerts its inhibitory effect on TGFβ signaling, remain somewhat open to interpretation. Without either loss of function and/or gain of function studies of all steps in the TGFβ signaling pathway, it is difficult to put these latter findings into the fibrosis context.

Calcium entry into cells via ion channels is highly cited in the literature as an essential process underlying fibroblast transition to a myofibroblast (10). Geiger and colleagues show that calcium is necessary but not sufficient to make a myofibroblast. Therefore, it remains possible that other mono or bivalent cations (Mg^{2+}, Na^{+}, and K^{+}) through ion channels are drivers of fibrosis or that other calcium channels contribute to a greater extent to the fibrotic process (11). In addition, it is not just a net increase in intracellular calcium, which drives myofibroblast differentiation, as difficult-to-detect timing of calcium increases, either transient, persistent, or cyclical, may condition the response (10). Similarly, the subcellular location of calcium oscillations, the extent of concomitant soluble profibrotic signals, and/or cross-talk with other channel-dependent and/or independent signals may also drive the fibrotic process (10, 12). Furthermore, TRP channels have intracellular domains, which may interact with other signaling cascades, as we have shown for TRPV4 in fibroblasts and macrophages (13–15). Future work that uncovers these key mechanisms will allow for more targeted therapy.

TRP channel cross-talk has been shown to modulate intracellular processes through channel–channel and/or protein–protein interactions as well as through posttranslational modifications in neurons (7). For example, coexpression of TRPA1 and TRPV1 is required for proper calcium-dependent neuronal functions (16, 17). Cofactors (e.g., Tmem100 [transmembrane protein 100] and prokineticins [PK1 and PK2]) have also been identified, which modulate both TRPA1 and TRPV1 neuropathic function (18). This suggests that targeting a binding partner to TRPA1–TRPV1 can potentially alter ion channel function or sensitivity. Although the phosphorylation of TRPA1 is less well described, there is evidence that TRPA1 interacts with a G-protein–coupled bradykinin receptor through activation of PKA and PKC (protein kinase A and C) (19–21).

The spectrum of nonspecific ion channel simulators spans the gamut, from varying temperature, osmolality, mechanical stress, endogenous inflammatory ligands, and synthetic and/or naturally occurring chemical stimuli. TRPA1 in the lung has primarily been implicated in airway diseases such as chronic cough, asthma, rhinorrhea, and chronic obstructive pulmonary disease. TRPA1 is ubiquitously expressed but primarily in sensory afferent nerves, which detect irritants and/or inhaled (e.g., cigarette smoke, pollutants, and chlorine) in the airway. Byproducts of pollutants, specifically exhaust particles, have been shown to agonize TRPA1 as a mechanism of cough exacerbation. Furthermore, asthmatic smooth muscle TRPA1 activation limits smooth muscle proliferation in the airway, implicating TRPA1 in associative studies or patients with asthma (22). Activation of TRPA1 has been directly associated with chronic rhinitis (23). The current study by Gieger and colleagues highlights the role of TRPA1 in parenchymal cells such as the

Support by NIH/NHLBI grants HL155064 (R.G.S) and HL158746 (M.A.O).

Originally Published in Press as DOI: 10.1165/rcmb.2022-0446ED on November 22, 2022

fibroblasts; however, others have shown pulmonary alveolar epithelial cell activation via the MAPKs and/or NF-κB pathways (24). Given the ubiquitous nature of its expression and its differential roles in a variety of cell types in the lung, which are constantly exposed to external stimuli, TRPA1 remains an intriguing but tricky target of modulation in respiratory diseases.

Many unanswered questions remain in the field of ion channel biology related to pulmonary fibrosis. When are the channels activated? Are there soluble activators released on epithelial injury, which initiate the fibrotic process? Do we seek to target the ion channel itself or its interacting partner? How do we target the cation flux in a cell-type and context-specific manner? Identification of the primary ion channel or interacting partner to target will be a significant advance in the field of pulmonary fibrosis. In the study by Geiger and colleagues, TRPA1 modulated myofibroblast differentiation not sufficiently through calcium or TGF-β1. It remains unknown how or if TRPA1 cooperates with other cation and/or calcium channels to mediate the fibrotic process *in vivo*. This paper, taken together with published work, sheds light on the possibility that multiple channels are orchestrated in the pathogenesis of pulmonary fibrosis. ■

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

Rachel G. Scheraga, M.D.
Respiratory Institute
Cleveland Clinic
Cleveland, Ohio

Mitchell A. Olman, M.D., M.A.
Lerner Research Institute
Cleveland Clinic
Cleveland, Ohio

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

References

1. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, *et al.*; ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788–824.
2. King TE Jr, Schwarz MI, Brown K, Tooze JA, Colby TV, Waldron JA Jr, *et al.* Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. *Am J Respir Crit Care Med* 2001;164:1025–1032.
3. Koivisto A-P, Belvisi MG, Gaudet R, Szallasi A. Advances in TRP channel drug discovery: from target validation to clinical studies. *Nat Rev Drug Discov* 2022;21:41–59.
4. Fallah HP, Ahuja E, Lin H, Qi J, He Q, Gao S, *et al.* A review on the role of TRP channels and their potential as drug targets_an insight into the TRP channel drug discovery methodologies. *Front Pharmacol* 2022;13:914499.
5. Rahaman SO, Grove LM, Paruchuri S, Southern BD, Abraham S, Niese KA, *et al.* TRPV4 mediates myofibroblast differentiation and pulmonary fibrosis in mice. *J Clin Invest* 2014;124:5225–5238.
6. Mukherjee S, Ayaub EA, Murphy J, Lu C, Kolb M, Ask K, *et al.* Disruption of calcium signaling in fibroblasts and attenuation of bleomycin-induced fibrosis by nifedipine. *Am J Respir Cell Mol Biol* 2015;53:450–458.
7. Talavera K, Startek JB, Alvarez-Collazo J, Boonen B, Alpizar YA, Sanchez A, *et al.* Mammalian transient receptor potential TRPA1 channels: from structure to disease. *Physiol Rev* 2020; 100:725–803.
8. Birrell MA, Belvisi MG, Grace M, Sadofsky L, Faruqi S, Hele DJ, *et al.* TRPA1 agonists evoke coughing in guinea pig and human volunteers. *Am J Respir Crit Care Med* 2009;180:1042–1047.
9. Geiger F, Zeitlmayr S, Staab-Weijnitz CA, Rajan S, Breit A, Gudermann T, *et al.* An inhibitory function of TRPA1 channels in TGF-β1-driven fibroblast to myofibroblast differentiation. *Am J Respir Cell Mol Biol* 2023;68:314–325.
10. Janssen LJ, Mukherjee S, Ask K. Calcium homeostasis and ionic mechanisms in pulmonary fibroblasts. *Am J Respir Cell Mol Biol* 2015;53:135–148.
11. Scruggs AM, Grabauskas G, Huang SK. The role of KCNMB1 and BK channels in myofibroblast differentiation and pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2020;62:191–203.
12. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* 2000;1:11–21.
13. Scheraga RG, Abraham S, Niese KA, Southern BD, Grove LM, Hite RD, *et al.* TRPV4 mechanosensitive ion channel regulates lipopolysaccharide-stimulated macrophage phagocytosis. *J Immunol* 2016;196:428–436.
14. Scheraga RG, Abraham S, Grove LM, Southern BD, Crish JF, Perelas A, *et al.* TRPV4 protects the lung from bacterial pneumonia via MAPK molecular pathway switching. *J Immunol* 2020;204: 1310–1321.
15. Grove LM, Mohan ML, Abraham S, Scheraga RG, Southern BD, Crish JF, *et al.* Translocation of TRPV4-PI3Kγ complexes to the plasma membrane drives myofibroblast transdifferentiation. *Sci Signal* 2019;12:eaau1533.
16. Sadofsky LR, Sreekrishna KT, Lin Y, Schinaman R, Gorka K, Mantri Y, *et al.* Unique responses are observed in transient receptor potential ankyrin 1 and vanilloid 1 (TRPA1 and TRPV1) co-expressing cells. *Cells* 2014;3:616–626.
17. Salas MM, Hargreaves KM, Akopian AN. TRPA1-mediated responses in trigeminal sensory neurons: interaction between TRPA1 and TRPV1. *Eur J Neurosci* 2009;29:1568–1578.
18. Weng H-J, Patel KN, Jeske NA, Bierbower SM, Zou W, Tiwari V, *et al.* Tmem100 is a regulator of TRPA1-TRPV1 complex and contributes to persistent pain. *Neuron* 2015;85:833–846.
19. Brackley AD, Gomez R, Guerrero KA, Akopian AN, Glucksman MJ, Du J, *et al.* A-kinase anchoring protein 79/150 scaffolds transient receptor potential A 1 phosphorylation and sensitization by metabotropic glutamate receptor activation. *Sci Rep* 2017;7:1842.
20. Meents JE, Fischer MJM, McNaughton PA. Sensitization of TRPA1 by protein kinase A. *PLoS One* 2017;12:e0170097.
21. Wang S, Dai Y, Fukuoka T, Yamanaka H, Kobayashi K, Obata K, *et al.* Phospholipase C and protein kinase A mediate bradykinin sensitization of TRPA1: a molecular mechanism of inflammatory pain. *Brain* 2008;131:1241–1251.
22. Balestrini A, Joseph V, Dourado M, Reese RM, Shields SD, Rougé L, *et al.* A TRPA1 inhibitor suppresses neurogenic inflammation and airway contraction for asthma treatment. *J Exp Med* 2021;218: e20201637.
23. Tóth E, Tornóczky T, Kneif J, Perkecz A, Katona K, Piski Z, *et al.* Upregulation of extraneuronal TRPV1 expression in chronic rhinosinusitis with nasal polyps. *Rhinology* 2018;56:245–254.
24. Ko H-K, Lin A-H, Perng D-W, Lee T-S, Kou YR. Lung epithelial TRPA1 mediates lipopolysaccharide-induced lung inflammation in bronchial epithelial cells and mice. *Front Physiol* 2020;11:596314.