lung inflation affect the degree of airway closure, impacting relationships between supine TAC and upright residual volume/TLC in COPD (2) and asthma (5). Although it is possible for lung models to estimate the posture-dependent effect (15), many physiological tests can be performed in both the upright and supine positions. A practical challenge for future HRCT studies is to reduce radiation exposure, enabling dynamic CT images to investigate the intermittent nature of airway closure in asthma, or the expansion dynamics of the thinned airway walls in COPD (2) and thickened airway walls in asthma (5). On both the image and lung function sides, we owe it to patients with lung disease to make every possible effort to extract as much structural information from the images we have, and to make the lung models that can help us interpret the structure–function link as realistic as possible. This is where morphometric and ventilation data obtained from the same patients (5) can be very helpful. ■

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References

1. Diaz AA, Valim C, Yamashiro T, Estépar RS, Ross JC, Matsuoka S, *et al*. Airway count and emphysema assessed by chest CT imaging predicts clinical outcome in smokers. *Chest* 2010;138:880–887.
2. Kirby M, Tanabe N, Tan WC, Zhou G, Obeidat M, Hague CJ, *et al*.; CanCOLD Collaborative Research Group; Canadian Respiratory Research Network; CanCOLD Collaborative Research Group, the Canadian Respiratory Research Network. Total airway count on computed tomography and the risk of chronic obstructive pulmonary disease progression: findings from a population-based study. *Am J Respir Crit Care Med* 2018;197:56–65.
3. McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, *et al*. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. *N Engl J Med* 2011;365: 1567–1575.
4. Dunican EM, Elicker BM, Gierada DS, Nagle SK, Schiebler ML, Newell JD, *et al*.; National Heart Lung and Blood Institute (NHLBI) Severe Asthma Research Program (SARP). Mucus plugs in patients with asthma linked to eosinophilia and airflow obstruction. *J Clin Invest* 2018;128:997–1009.
5. Eddy RL, Svenningsen S, Kirby M, Knipping D, McCormack DG, Licskai C, *et al*. Is computed tomography airway count related to asthma severity and airway structure and function? *Am J Respir Crit Care Med* 2020;201:923–933.
6. Zha W, Kruger SJ, Cadman RV, Mummy DG, Evans MD, Nagle SK, *et al*. Regional heterogeneity of lobar ventilation in asthma using hyperpolarized helium-3 MRI. *Acad Radiol* 2018;25:169–178.
7. Polak AG, Lutchen KR. Computational model for forced expiration from asymmetric normal lungs. *Ann Biomed Eng* 2003;31:891–907.
8. Foy BH, Soares M, Bordas R, Richardson M, Bell A, Singapuri A, *et al*. Lung computational models and the role of the small airways in asthma. *Am J Respir Crit Care Med* 2019;200:982–991.
9. Milne S, Jetmalani K, Chapman DG, Duncan JM, Farah CS, Thamrin C, *et al*. Respiratory system reactance reflects communicating lung volume in chronic obstructive pulmonary disease. *J Appl Physiol (1985)* 2019;126:1223–1231.
10. Foy B, Kay D, Siddiqui S, Brightling C, Paiva M, Verbanck S. Increased ventilation heterogeneity in asthma can be attributed to proximal bronchioles. *Eur Respir J* 2019;1901345.
11. Verbanck S, Ghorbaniasl G, Biddiscombe MF, Dragojlovic D, Ricks N, Lacor C, *et al*. Inhaled aerosol distribution in human airways: a scintigraphy-guided study in a 3D printed model. *J Aerosol Med Pulm Drug Deliv* 2016;29:525–533.
12. Verbanck S, King GG, Paiva M, Schuermans D, Vanderhelst E. The functional correlate of the loss of terminal bronchioles in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2018; 197:1633–1635.
13. Verbanck S, King GG, Zhou W, Miller A, Thamrin C, Schuermans D, *et al*. The quantitative link of lung clearance index to bronchial segments affected by bronchiectasis. *Thorax* 2018;73:82–84.
14. Donovan GM, Elliot JG, Green FHY, James AL, Noble PB. Unraveling a clinical paradox: why does bronchial thermoplasty work in asthma? *Am J Respir Cell Mol Biol* 2018;59:355–362.
15. Bell AJ, Foy BH, Richardson M, Singapuri A, Mirkes E, van den Berge M, *et al*. Functional CT imaging for identification of the spatial determinants of small-airways disease in adults with asthma. *J Allergy Clin Immunol* 2019;144:83–93.

FSTL-1: A New Player in the Prevention of Emphysema

Emphysema is an incurable destructive lung disease that causes impairment in gas exchange, gas trapping, hyperinflation, and ultimately shortness of breath. Cigarette smoking is a major cause of emphysema, but there are also less common genetic causes of emphysema, such as alpha-1 antitrypsin deficiency (1). The mechanisms underlying the induction and progression of emphysema are believed to involve an imbalance in lung proteases and antiproteases, chronic inflammation and oxidative stress, alveolar wall cell death, and failure of alveolar wall maintenance (2, 3). A better understanding of the cellular and molecular mechanisms that drive emphysema may lead to novel therapeutic strategies to prevent its development or halt its progression, resulting in better health outcomes for patients.

There is a growing body of evidence showing that FSTL-1 (follistatin-like 1) plays an important role in lung development and respiratory diseases, including asthma and pulmonary fibrosis (4–8). In a study presented in this issue of the *Journal*, Henkel and colleagues (pp. 934–945) examined the consequences of reduced FSTL-1 expression on postnatal lung homeostasis (9). For this

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purpose, they used mice with reduced *Fstl1* gene expression, termed FSTL-1 hypomorphic (FSTL-1 Hypo) mice. The authors found that FSTL-1 Hypo mice spontaneously developed emphysema and that pulmonary function was impaired. Micro–computed tomography scanning showed that FSTL-1 Hypo mice had increased lung volumes and decreased lung density. Collectively, these findings indicate that reduced FSTL-1 expression was sufficient to cause the mice to develop histologic, functional, and radiographic findings consistent with pulmonary emphysema. A very interesting finding in this study was that FSTL-1–dependent emphysema was not exacerbated by chronic cigarette smoke exposure, suggesting that FSTL-1 deficiency protects mice from cigarette smoke–induced emphysema. Given that emphysema is a clinical condition of cigarette smoke–induced chronic obstructive pulmonary disease (COPD), and that novel treatments are desperately needed, it would have been worth expanding on this intriguing aspect of the study.

Because FSTL-1 protects against emphysema, the authors went on to investigate the cellular sources of FSTL-1 in the lung. Interestingly, FSTL-1 was highly expressed in endothelial and mesenchymal cells, both significantly more so than in epithelial and immune cells. To determine whether murine FSTL-1 expression correlated with human lung cell FSTL-1 expression, the authors analyzed data from the Lung Gene Expression Analysis Web Portal and found that in 20-month-old human lung tissue, FSTL1 expression was evident in the endothelium, epithelium, and (most highly) mesenchyme. Together, these observations identify endothelial and mesenchymal cells as the primary producers of FSTL-1 in the postnatal lung.

RNA sequencing was used to determine the mechanism(s) by which FSTL-1 Hypo mice develop emphysema. This identified 33 genes that were significantly differentially expressed between wild-type (WT) and FSTL1 Hypo mice, irrespective of cigarette smoke exposure. Of particular note was that several gene regulation and macrophage antiinflammatory genes were differentially expressed in an FSTL-1–dependent manner, including the nuclear orphan receptor Nr4a1, also known as Nur77. Using qRT-PCR, the authors validated this finding by showing that Nr4a1 gene expression was significantly decreased in FSTL-1 Hypo mice, as well as cigarette smoke–exposed WT mice, compared with WT mice exposed to room air. They then used fluorescence-activated cell sorting to show that reduced FSTL-1 expression was associated with decreased Nr4α1 expression and $Nur77^{+}$ staining within the lung, which corresponded with increases in myeloid cell abundance and reduced myeloid Nur77 positivity. Taken together, these data suggest that FSTL-1 may act directly on macrophages to influence Nr4α1/Nur77 function.

Because lung cell apoptosis is a known cause of emphysema (2, 3), and Nr4α1 and FSTL-1 have been shown to influence cell survival via apoptosis (10, 11), the authors investigated whether endothelial or epithelial cell apoptosis could explain the observed FSTL-1 Hypo phenotype. Surprisingly, FSTL-1 Hypo mice showed no difference in the percentage of apoptosis in endothelial, epithelial, or mesenchymal cells, suggesting that this mechanism did not account for the observed emphysema in FSTL-1 Hypo mice.

Genome-wide association studies have provided new insights into the molecular mechanisms of COPD and lung function (12, 13), which can be used to develop new drugs against molecular targets and inform population-based preventive strategies for targeting these previously unidentified molecular pathways. To investigate whether FSTL1 SNPs were associated with COPD-related phenotypes, the authors analyzed genotype data from non-Hispanic white participants in the COPDGene project. The authors show for the first time that genetic polymorphisms in the FSTL1 locus may influence COPD and lung function in a subset of individuals.

There are some potential limitations to this study that bear some discussion. First, it would have been worth exploring whether Nr4a1 can rescue the FSTL-1 Hypo emphysema phenotype. Second, to investigate whether FSTL1 SNPs were associated with COPD-related phenotypes, the authors analyzed genotype data from non-Hispanic white participants in the COPDGene project. It would have been worth examining FSTL1 SNPs in other cohorts and populations, as well as measuring FSTL-1 levels/expression in other sample types (e.g., blood, serum, and lung tissue) from COPD study populations. However, despite these potential limitations, this valuable study will inform and prompt further studies in the field to verify and expand on the outcomes of the present study.

In summary, Henkel and colleagues have identified a novel role of FSTL-1 in protecting against the development of emphysema in mice, which is independent of cigarette smoke exposure. This appears to be achieved by FSTL-1 affecting NF-κB (nuclear factor-κB) signal transduction in macrophages via modulation of Nr4α1. Although the exact nature of an FSTL-1/Nr4a1/NF-κB pathway in human emphysema remains to be defined, this study has important clinical implications, as it may pave the way for novel therapeutics that can prevent or halt the progression of emphysema. ■

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References

1. Singh D, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, *et al*. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD Science Committee report 2019. *Eur Respir J* 2019;53:1900164.
2. Sharafkhaneh A, Hanania NA, Kim V. Pathogenesis of emphysema: from the bench to the bedside. *Proc Am Thorac Soc* 2008;5:475–477.
3. Goldklang M, Stockley R. Pathophysiology of emphysema and implications. *Chronic Obstr Pulm Dis (Miami)* 2016;3:454–458.
4. Dong Y, Geng Y, Li L, Li X, Yan X, Fang Y, *et al*. Blocking follistatin-like 1 attenuates bleomycin-induced pulmonary fibrosis in mice. *J Exp Med* 2015;212:235–252.
5. Geng Y, Dong Y, Yu M, Zhang L, Yan X, Sun J, *et al*. Follistatin-like 1 (Fstl1) is a bone morphogenetic protein (BMP) 4 signaling antagonist in controlling mouse lung development. *Proc Natl Acad Sci USA* 2011; 108:7058–7063.
6. Miller M, Esnault S, Kurten RC, Kelly EA, Beppu A, Das S, *et al*. Segmental allergen challenge increases levels of airway follistatin-like 1 in patients with asthma. *J Allergy Clin Immunol* 2016;138:596–599.e4.
7. Miller M, Beppu A, Rosenthal P, Pham A, Das S, Karta M, *et al*. Fstl1 promotes asthmatic airway remodeling by inducing oncostatin M. *J Immunol* 2015;195:3546–3556.

8. Liu T, Liu Y, Miller M, Cao L, Zhao J, Wu J, *et al*. Autophagy plays a role in FSTL1-induced epithelial mesenchymal transition and airway remodeling in asthma. *Am J Physiol Lung Cell Mol Physiol* 2017;313: L27–L40.
9. Henkel M, Partyka J, Gregory AD, Forno E, Cho MH, Eddens T, *et al*. FSTL-1 attenuation causes spontaneous smoke-resistant pulmonary emphysema. *Am J Respir Crit Care Med* 2020;201: 934–945.
10. Ipseiz N, Uderhardt S, Scholtysek C, Steffen M, Schabbauer G, Bozec A, *et al*. The nuclear receptor Nr4a1 mediates anti-inflammatory effects of apoptotic cells. *J Immunol* 2014;192: 4852–4858.
11. Ni X, Cao X, Wu Y, Wu J. FSTL1 suppresses tumor cell proliferation, invasion and survival in non-small cell lung cancer. *Oncol Rep* 2018; 39:13–20.
12. Shrine N, Guyatt AL, Erzurumluoglu AM, Jackson VE, Hobbs BD, Melbourne CA, *et al*.; Understanding Society Scientific Group. New genetic signals for lung function highlight pathways and chronic obstructive pulmonary disease associations across multiple ancestries. *Nat Genet* 2019;51:481–493. [Published erratum appears in *Nat Genet* 51:1067.]
13. Sakornsakolpat P, Prokopenko D, Lamontagne M, Reeve NF, Guyatt AL, Jackson VE, *et al*.; SpiroMeta Consortium; International COPD Genetics Consortium. Genetic landscape of chronic obstructive pulmonary disease identifies heterogeneous cell-type and phenotype associations. *Nat Genet* 2019;51: 494–505.

TMEM16A Potentiators: Is There a Need for New Modulators in Cystic Fibrosis?

On October 21, 2019, the U.S. Food and Drug Administration approved a highly effective triple combination therapy (elexacaftor, tezacaftor, and ivacaftor) for patients with cystic fibrosis (CF) with at least one copy of the F508del mutation in CFTR (CF transmembrane conductance regulator). This combination therapy improved lung function, sweat chloride, weight, and quality of life in an unprecedented fashion for a folding mutation (1, 2). Thus, the currently available highly active modulators have the potential to reach ~90% of the population of patients with CF. This raises the question whether additional therapies for CF or other airway diseases related to CFTR dysfunction are still needed.

Given the remaining patients with CF without current highly effective treatment modalities, patients who cannot tolerate or who respond poorly to available modulators and the uncertainty of the treatment efficacy over time, the answer is a resounding yes. The question then is which mechanisms to target. Because lung disease is a major contributor to morbidity and mortality, a main aim of CFTR modulators is to hydrate mucus by restoring airway surface liquid volume to facilitate mucociliary clearance (MCC). Homeostasis of airway surface liquid is tightly regulated and maintained by the balance of ion channel activities in the airway epithelium, including chloride secretion by CFTR and calcium-activated chloride (CaCC) channels, potassium secretion by large-conductance calcium-activated and voltage-dependent potassium (BK) channels, and sodium absorption by the epithelial sodium (ENaC) channels (3, 4). Ion channels that compensate for defective CFTR therefore represent an important therapeutic target group.

Restoring airway surface hydration in CF by modulating ion channel function alternative to CFTR has shown promise as a therapeutic option *in vitro* and in animal studies, but strategies targeting these proteins had limited success in clinical trials. So far, no therapeutics have been developed for BK channel activation. Approaches to down-regulate the expression and/or function of ENaC have been proposed as a means to improve mucus hydration. Unfortunately, prototypical ENaC blockers such as amiloride showed no clinical efficacy, whereas amiloride analogs and other approaches provided thus far no measurable clinical benefit (5, 6), possibly because of low dosing to avoid toxicity.

Therapeutic strategies to enhance apical chloride secretion alternative to CFTR have been attempted even before the molecular identification of TMEM16A (transmembrane member 16A), also known as ANO1 (anoctamin 1), as one of the CaCCs (7–9). Early studies *in vitro* and in normal subjects provided evidence that stable uridine-5′-triphosphate analogs stimulate CaCC via increases in cytosolic calcium and improve MCC (10, 11). However, these selective P2Y2 purinergic receptor agonists also stimulated mucin secretion (12) and ultimately failed to demonstrate any clinical benefit in the large phase 3 clinical trial TIGER-2 (Transport of Ions to Generate Epithelial Rehydration).

TMEM16A is a highly conserved member of a larger family of proteins that comprise Ca^{2+}-dependent ion channels and phospholipid scramblases. Although TMEM16A is thought to be a significant contributor to CaCCs in the airways, the exact function of TMEM16A in airway disease remains controversial. This largely stems from findings that TMEM16A is expressed at low levels in the airway epithelium under normal conditions, and that its expression is highly induced by proinflammatory cytokines, including IL-4 and IL-13 that drive goblet cell hyperplasia (7, 13). Furthermore, the possible prosecretory effect of TMEM16A on mucin secretion (13) led to speculations that augmentation of TMEM16A activity may have adverse effects on MCC in CF.

In this issue of the *Journal*, Danahay and colleagues (pp. 946–954) describe the identification of a novel TMEM16A potentiator, ETX001, that enhances anion secretion and improves MCC both in primary CF bronchial epithelial cells *in vitro* (unstimulated and stimulated with IL-13) and in an *in vivo* sheep model of CF-like airway disease (14). ETX001 failed to stimulate TMEM16A activity in the absence of calcium, but rather

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