Epigenetic Links to Airway Smooth Muscle Proliferation

The global morbidity and mortality rates of chronic obstructive pulmonary disease (COPD) are increasing and current antiinflammatory drugs have limited efficacy. COPD is characterized by persistent airway inflammation, which causes related histological and cytological changes in the airway structure over time that are referred to as "airway remodeling." Airway remodeling may lead to thickening of the airway wall, thereby promoting airway narrowing and airflow limitation (1). Remodeling includes subepithelial fibrosis, goblet cell hyperplasia, submucosal gland hypertrophy, and an increase in the airway smooth muscle (ASM) layer thickness (2). The increase in ASM thickness results from either an increase in the number of ASM cells (ASMCs) due to proliferation and hyperplasia or an increase in the volume of each individual ASMC (hypertrophy), or a combination of both. Indeed, increased ASM mass may be a key feature of airway remodeling (3) and is associated with the decreased lung function observed in COPD (4). However, the current mechanism of ASM layer thickening in patients with COPD is still unclear.

ASMCs exhibit a shift to a more proliferative phenotype in response to external stimuli such as growth factors and tobacco smoke exposure (5, 6). This phenotype plasticity, including altered contractility and relaxation, cell proliferation and migration, and secretion of inflammatory mediators and matrix metalloproteinases, is associated with the airway remodeling in COPD (6). However, the mechanisms responsible for ASM proliferation in COPD are not well defined.

Calcium (Ca^{2+}) signaling plays an important role in the regulation of ASMC functions such as proliferation, contraction, and cytokine secretion. Store-operated calcium entry (SOCE) is the major pathway for Ca^{2+} influx and is activated by depletion of the intracellular sarcoplasmic reticulum Ca^{2+} stores (7). Previous studies have demonstrated that regulation of calcium influx contributes to ASMC proliferation *in vitro* (7–9). In addition, ASMC proliferation has been linked to increased expression of cell cycle regulators and proliferation markers such as proliferating cell nuclear antigen (PCNA), cyclin E, and cyclin D1 (6, 10). Thus, control of ASMC proliferation via the regulation of critical pathways might serve as a promising therapeutic strategy for COPD.

Long noncoding RNAs (lncRNAs) are a major class of ncRNAs with lengths greater than 200 nt and have been shown to play essential roles in many cell biological processes, including cell development, growth, and migration in various diseases, by modulating DNA, RNA, and protein (11). Abnormal expression of lncRNAs has been associated with smokers with COPD (12). Furthermore, lncRNAs such as *SCAL1* (smoke- and cancer-associated lncRNA-1) and *TUG-1* (taurine-upregulated gene 1) have been implicated in the pathogenesis of COPD (13, 14). LncRNAs are also known to regulate primary pulmonary ASMCs (15). Using RNA sequencing (RNA-seq), Chen and colleagues previously identified 725 lncRNAs in rat pulmonary ASMCs, 95 of which were expressed differentially after stimulation with the proliferative agent PDGF-BB (platelet-derived growth factor BB) (15). Recent studies have also indicated that lncRNAs can modulate ASMC function in asthma. The lncRNA *PVT1* (plasmacytoma variant translocation 1) plays an important role in controlling both proliferation and IL-6 release in primary human ASMCs and is associated with the aberrant ASMC phenotype observed in patients with severe asthma. *PVT1* might be a useful target for reducing airway remodeling in these subjects (16). Other lncRNAs, such as *BCYRN1* (brain cytoplasmic RNA 1), promote the proliferation and migration of rat ASMCs in asthma by increasing the expression of TRPC1 (transient receptor potential 1) (17). Moreover, the lncRNA *GAS5* (growth arrest-specific transcript 5) enhances ASMC proliferation in asthma by controlling the miR-10a/BDNF (brain derived neurotrophic factor) signaling pathway (18).

In this issue of the *Journal*, Zheng and colleagues (pp. 584–596) report on a study of the differential expression of lncRNAs in resected lung tissue from nonsmokers, smokers, and smokers with COPD using RNA-seq (19). They performed hierarchical clustering on 46 significantly differentially expressed lncRNAs and constructed a coding–noncoding gene coexpression network to explore the relationships between lncRNAs and significantly changed genes in patients with COPD compared with smokers without COPD. Because *ENST00000460164.1* (*COPDA1*) was significantly upregulated in lung tissue from patients with COPD compared with smokers without COPD and was closely related to its target gene *MS4A1*, which plays an important role in regulating cell activation and proliferation, the authors focused on *COPDA1* and *MS4A1* in this research. The study revealed that *COPDA1* was expressed in human ASMCs and facilitated their growth by targeting *MS4A1* to increase SOCE and the ionized calcium concentration in the cells. This is an innovative mechanism for the proliferation of ASM in COPD, and their study provides a novel potential target for COPD therapy from the genetic perspective of lncRNAs.

However, some limitations as well as strengths of the current report should be noted. Although the initial RNA-seq analysis was performed in resected lung from only 18 subjects (six per group), and the authors verified *COPDA1* expression in subjects with COPD and smokers without COPD by qPCR and validated the expression in an additional 53 lung tissue samples from a distinct cohort, they did not assess the levels of *COPDA1* according to Global Initiative for Chronic Obstructive Lung Disease stage or determine whether active or ex-smoking affected expression levels. Because lncRNAs have several differences compared with mRNAs, including *1*) possessing a more pronounced tissue-specific pattern than protein-coding genes, *2*) being more stable against

degradation, *3*) having a longer half-life in biological samples, and *4*) being more readily and reproducibly detected (20), it would have been useful to determine whether *COPD1A* could be detected in noninvasive liquid biosamples such as serum, sputum, and exhaled breath condensates. This would enable more effective clinical validation in other cohorts using accessible samples. Furthermore, future work with the cigarette smoke–induced mouse COPD model and with transgenic/knockout animals, or perturbation experiments to further investigate the effects of the lncRNA *COPDA1* and its associated signal pathway in the airway remodeling of COPD, would extend the scope of the study.

Current therapy for COPD has only limited disease-modifying effects. Recently, Li and colleagues demonstrated that silencing *PFAL* (pulmonary fibrosis-associated lncRNA) using AAV5 (adeno-associated virus 5) carrying shRNA against the lncRNA could alleviate lung fibrosis in a bleomycin-induced mouse model (21). Although there are no reports concerning the targeting of lncRNAs for airway remodeling therapeutics, the study by Zheng and colleagues suggests that targeting the lncRNA *COPDA1* may reverse airway remodeling caused by ASMC proliferation in COPD. ■

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