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More Than Meets the Eye: Cigarette Smoke Induces Genomic Changes in the Small Airway Epithelium Independent of Histologic Changes

Cigarette smoke–induced lung diseases, including lung cancer and chronic obstructive pulmonary disease (COPD), are leading causes of morbidity and mortality. The airway "field of injury" hypothesis suggests that exposure to a disease or environmental insult, such as cigarette smoke, leads to molecular alterations throughout the whole respiratory system, and that these alterations occur even in the absence of histologic changes. This concept, well developed in the cancer literature, suggests exposure-associated molecular alterations can be measured in histologically normal airway epithelium by gene expression profiling (1). These genomic signatures can then be used both to gain insights into disease mechanisms and to generate biomarkers for disease onset, progression, prognosis, and treatment.

In COPD, the earliest pathological changes appear to occur in the small airways (2–4). Cigarette smoke induces squamous cell metaplasia and mucous cell hyperplasia in the small airway epithelium (SAE) (5, 6). Further, there is evidence of decreased SAE repair (7), suggesting a detrimental effect of cigarette smoke on basal cells (BCs), the airway stem or progenitor cells (8). Although cigarette smoke–induced, SAE-specific molecular alterations have been identified (9–11), whether these molecular alterations precede these early pathologic changes is less well studied. The progression of this early injury to the heterogeneous pathologic changes in COPD, including emphysema and bronchitis, is also poorly understood, especially in former smokers.

In this issue of the *Journal*, Yang and colleagues (pp. 340–352) advance our understanding of the cigarette smoke–induced airway field of injury (12). They focus on molecular alterations induced in the SAE compared with the larger bronchi, leveraging the group's small airway brushing collection technique. By comparing global gene expression profiles of the large and small airway epithelium from healthy control patients, they developed proximal and distal airway transcriptome signatures (P- or D-signatures). Using immunohistochemistry, the authors established that the genomic differences between regions was not simply a result of distinct compositions of known cell types by demonstrating that certain proximal gene expression markers are expressed by ciliated cells, a cell type also abundant in the distal airways in which these genes have lower expression. They next compared the SAE gene expression of smokers with and without COPD with that of nonsmokers. Smokers exhibited a down-regulation of ~50% of D-signature genes compared with nonsmokers, whereas P-signature genes were up-regulated. These smoking-induced SAE molecular alterations were termed "distal-to-proximal repatterning." The study further shows that the degree of proximalization was associated with lung function (FEV_1/FVC ratio) and age in healthy smokers, suggesting these genomic lesions have functionally measurable consequences.

As pathway analysis revealed EGFR as a major upstream regulator of the P-signature genes, the authors demonstrated evidence for its relevance *in vitro* by culturing primary human BCs at an air–liquid interface. Treatment of proximal airway BC cultures with an EGFR inhibitor decreased the expression of P-signature genes and increased D-signature genes. SAE BC cultures exhibited opposite changes when treated with EGF. EGF was further found to be up-regulated in the SAE of smokers, a finding reproduced by exposing cultures to cigarette smoke extract.

The changes induced *in vitro* by cigarette smoke extract support the concept that SAE proximalization represents early

injury from smoking. The observation on immunohistochemistry that one P-signature gene, *UPK1B*, is induced in SAE ciliated cells from smokers, in areas free from squamous metaplasia and mucous cell hyperplasia, further suggests SAE proximalization precedes the development of histologic lesions.

Taken together, Yang and colleagues report an extensive SAE transcriptome analysis, which identifies proximalization of distal airways as at least partially a result of cigarette smoke–induced EGF signaling. However, as a result of the study design, it is difficult to determine which of the observed effects are specifically associated with COPD pathogenesis. Although they did use SAE samples from current smokers with and without COPD, samples obtained from participants with COPD had higher smoke exposure (significantly higher pack-years and slightly higher nicotine and cotinine levels, although of unclear statistical significance). The COPD participants were also older, which was important, as age was associated with increased proximalization of the SAE in healthy smokers. Any inferences made specifically about COPD are thus confounded by smoke exposure and age. Furthermore, no former smokers or never smokers with COPD were included in the analysis. Additional studies are necessary to determine which, if any, of these proximalization-associated alterations are reversed by smoking cessation. Nonetheless, this study provides a deeper understanding of how cigarette smoke exposure regionally affects the airway field of injury, which is important for all smoking-related lung diseases.

The underlying mechanism of this SAE proximalization remains unknown. Nicotine, the most well-characterized cigarette ingredient, may alter airway BC proliferation and differentiation (13). However, the detrimental effects of the other toxic components of cigarette smoke should not be neglected. Noncoding RNA (e.g., microRNA) regulation, as well as epigenetic and microbiome alterations, may all influence exposure-related gene expression alterations. Authors from the current study previously identified SAE alterations in microRNA expression between healthy smokers and nonsmokers (14). DNA methylation has also been reported to be altered in SAE of COPD, which was linked to altered gene expression (15). A recent study reported a decreased diversity of microbiota derived from lower airways in COPD (16), and although unique smoking-induced changes in the distal airway microbiome have not been identified, one might speculate that such changes occur secondary to smoke exposure or an altered SAE in the development of COPD.

Individual variability in genetics, epigenetics, and the microbiome, in addition to the intensity of smoking and exposure to airborne pollutants, may also explain the striking heterogeneity observed in the degree of smoking-dependent proximalization of the SAE. An understanding of this heterogeneity has the potential to lead to the development of biomarkers for COPD risk and precision therapies.

Between proximalization of the SAE and translation to the clinic, there remain important gaps to fill. Although there is indirect evidence that repatterning is an early smoking-dependent lesion occurring before the traditionally observed histologic changes, proximalization might also or instead be a separate type of injury in a subgroup of smokers. Longitudinally collected airway brushings and samples from healthy and diseased areas of lung are required to establish repatterning as a precursor lesion. Employing matched samples from the distal and proximal compartments may also be crucial (as opposed to each sample from a distinct participant as in this study) to control for the heterogeneity identified across participants. If SAE proximalization is a precursor to COPD, the implications for treatment will be predicated in part on knowing whether the early changes reverse on smoking cessation. Finally, if proximalization is indeed an early form of smoking-induced injury that can progress to COPD, therapeutic strategies will likely need to target molecules downstream of EGFR or in the other pathways enriched in the proximal airway signature (e.g., oxidative stress and extracellular matrix-associated pathways), as EGFR inhibition in COPD was not successful in one randomized controlled trial (17). ■

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On the "TRAIL" of a Killer: MMP12 in Lung Cancer

Lung cancer continues to be the main cause of cancer death worldwide. Despite advances in surgical approaches, new chemotherapeutic agents, and novel screening techniques, the 5-year survival rate for lung cancer remains dismal (1). Failure of early tumor detection represents an important barrier to overcome in the battle against lung cancer, while continuing exposure to tobacco globally; the emergence of e-cigarettes, which might promote smoking in young generations; and the massive effect of biomass exposure in developing countries will undoubtedly extend the grasp of this killer on our society for decades to come. Despite the above, several recent discoveries raise optimism regarding our ability to slow down, if not eliminate, this threat. These include the identification of genetic mutations capable of driving tumorigenesis (e.g., EGFR) that have led to the development of effective and safe therapeutic interventions (2), the unveiling of the potent antitumor effects resulting from the unleashing of the host's immune system via checkpoint inhibitors (3), and the recognition that early screening of lung cancer with low-dose computer tomography in at-risk individuals may improve survival (4). In the end, however, one of our biggest challenges resides in addressing the following question: How do we kill lung cancer cells while protecting normal cells? Studies directed at answering this question are likely to accelerate discovery and lead to the generation of novel approaches to lung cancer.

In this issue of the *Journal*, Dandachi and colleagues (pp. 353–363) provide further clues into this area by exploring the antitumor effects of matrix metalloproteinase-12 (MMP12) (5). Also known as metalloelastase, MMP12 is a member of the family of matrix metalloproteinases (MMPs) characterized by their ability to degrade extracellular matrices (6). MMP12 has been implicated in smoking-related emphysema (7) and bacterial killing (8), but its exact role in cancer, especially in lung cancer, remains uncertain. In murine models, MMP12 is protective against tumor progression (9, 10), and this activity has been ascribed to the generation of anti-angiogenic peptides (11). In their study, Dandachi and colleagues describe another mechanism by which MMP12 exerts its anticancer effects (5).

The work was prompted by experiments showing that the co-culture of tumor cells with wild-type peritoneal macrophages blunted [^{3}H]-thymidine incorporation in A549 cells, whereas peritoneal macrophages harvested from MMP12-deficient animals failed to do so. Subsequently, they localized the effect of MMP12 to its 23-kD carboxy-terminal domain (CTD); its catalytic domain had no effect. The activity was further localized to a smaller CTD fragment, termed SR20, a fragment previously shown to enhance bacterial killing by macrophages (8). Because SR20 did not affect cell cycle progression, experiments were directed at investigating apoptosis. This work led to the discovery that SR20 travels to the nucleus and binds to DNA sequences upstream of the gene encoding for tumor necrosis factor– related apoptosis-inducing ligand (TRAIL), as well as its receptor, death receptor 4. TRAIL is a type II transmembrane and soluble polypeptide that triggers apoptotic cell death by binding to death receptors 4 (DR4) and 5 (DR5), which, in turn, activate caspase-8 and/or caspase-9 leading to caspase-3 cleavage and, subsequently, apoptosis (12). Simultaneously, SR20 decreased the levels of antiapoptotic proteins such as phosphor-Bcl-2-associated death promoter (pBad), as well as prosurvival proteins such as NF-κB and AKT, while increasing proapoptotic proteins such as Bad and Bcl-xS (Figure 1). The ability of SR20 to affect several arms of the apoptosis pathway in tumor cells highlights its potential effectiveness if used in therapeutics.

Three critical observations are worth highlighting from this work. First, CTD and SR20 induced apoptosis in both human (e.g., A549) and murine tumor cells, while not affecting normal lung epithelial cells. This is of utmost importance, as it emphasizes that the proapoptotic pathways affected by TRAIL are relevant in tumor cells and not in normal lung cells, thereby unveiling a target for intervention that has limited effect on host cells. Unfortunately, tumors are often resistant to TRAIL-related apoptosis through down-regulation of death receptor expression, overexpression of decoy receptors, or alterations in the downstream signals triggered by ligation of TRAIL receptors (13, 14). SR20 might be able to overcome these antiapoptotic mechanisms.

Second, MMP12 exerted its antitumor effects via a noncatalytic pathway. In general, MMPs exert many of their biological effects through the degradation of fibronectin, collagens, and other extracellular matrices. In doing so, these proteinases facilitate the migration of cells through basement membranes, generate chemotactic gradients, and release growth factors from the matrix environment.