Differential Methylation of Chronic Obstructive Pulmonary Disease Lung Macrophage Genes Sheds Light on Disease Pathogenesis

Altered DNA methylation has been implicated in a variety of human diseases, including respiratory diseases such as asthma, idiopathic pulmonary fibrosis, and chronic obstructive pulmonary disease (COPD) (1). DNA methylation, which involves the reversible transfer of a methyl group to the fifth carbon of a cytosine nucleotide followed by a guanine (CpG; p is for the phosphate connecting the bases!), can impact gene expression through mechanisms such as altering local chromatin landscape and recruiting or blocking relevant transcription factors at the locus. Illumina has developed successive genotyping-based arrays to assay methylation at CpGs across the genome. The most recent platform, the EPIC chip, assays ~850,000 CpGs with a focus on regulatory elements and CpG islands. These clusters of CpGs often overlap with gene promoters, show variable methylation, and often show an inverse correlation between methylation concentrations and the expression of related genes. Profiling the methylome allows for biological discovery as well as the identification of biomarkers related to the disease. Furthermore, modification of methylation of specific genomic regions is of major interest for pharmaceutical development, and we've seen the first wave of epigenetic therapeutics emerge (2).

In this issue of the *Journal*, Ström and colleagues (pp. 638–647) profile DNA methylation using the Illumina EPIC chip in BAL cells obtained from 18 subjects with COPD and 15 controls with normal lung function (3). While previous studies have investigated DNA methylation in COPD blood (4), buccal mucosa (5), and lung tissue (6), this is the first study performed using BAL, which contains immune cells present in bronchoalveolar regions. A recent study of metabolomic associations with COPD found that BAL contained many more compounds that were associated with measured COPD phenotypes than matched plasma samples (7), underscoring the importance of profiling cells from the target tissue. Ström and colleagues identified 1,155 differentially methylated positions (DMPs) between COPD and control samples. DMPs represent differentially methylated single CpGs and are enriched for CpG islands, biological pathways such as cellular senescence and structures including adherens and tight junctions, and macrophage-related genes, consistent with macrophages making up the majority of BAL cells. Despite highly significant *P* values, the majority of the DMPs have small effect sizes; the biological relevance of small changes in methylation is unclear at this time. However, the authors do identify significant CpGs with large DNA methylation changes that map to genes known to be involved in COPD, such as *SCNN1A* (sodium channel epithelial 1 subunit α) and novel genes such as *NOB1* (BUB1 binding protein 1 homolog) and *TFAP2A* (transcription factor AP-2 α). To assess the impact of DNA methylation changes on gene expression, the authors looked at correlations with genes within 250 kb in another BAL dataset (8), as their assessments did not include transcriptomic data. Correlations with gene expression were found for 79 DMPs, with the strongest correlations found for genes that included *CPD* (carboxypeptidase D) and *FLI1* (fli-1 proto-oncogene, ETS transcription factor). The authors also examined whether DMPs overlap with previously reported COPD genome-wide association signals. They asked whether any DMPs were within 500 kb of 82 SNPs identified in a genome-wide association meta-analysis (9) and identified 447 such DMPs. Of these, 10 DMPs were annotated to the same gene as the SNP, and 3 DMPs were within the gene *ADGRG6* (adhesion G protein-coupled receptor G6).

Illumina methylation array data can also be used to estimate epigenetic age using epigenetic clocks (10). These algorithms use a subset of CpGs assayed on Illumina arrays shown to be highly correlated with chronological age to predict epigenetic age. Accelerated epigenetic aging is often observed in disease (11). However, the authors report that they did not observe a differential effect of epigenetic aging in COPD. This is surprising given previous reports of associations between epigenetic aging and COPD. Breen and colleagues reported a significant association between accelerated epigenetic age at baseline and incident COPD in whole blood from the longitudinal KORA (Cooperative Health Research in the Region of Augsburg) cohort (12), while Hillary and colleagues found an association between accelerated epigenetic age and COPD in whole blood from Generation Scotland (13). Further studies should be done to confirm that epigenetic aging is not accelerated in COPD BAL.

The main strength of the study is the use of cells from patients in the OLIN (Obstructive Lung Diseases in Northern Sweden) cohort, allowing for close matching of COPD and control groups by age, sex, and body mass index, demographics that often confound lung tissue studies. There are also key limitations to the analyses performed in the article. Only 33 individuals were included in the analysis, which likely reflects the difficulty of obtaining BAL in patients with COPD. Furthermore, it is not known how generalizable the findings of this study are due to a lack of replication in other populations. Additionally, transcriptomic data are not available from this cohort, limiting the ability to integrate epigenomic and transcriptomic datasets. It would be interesting to explore the overlap of BAL with peripheral blood and whole lung tissue results to determine if any signals are consistent or correlated.

Ultimately, the authors identify methylation in previously implicated genes in COPD, as well as interesting novel signals. The large number of novel targets, especially considering the small sample size, highlights the utility of assaying methylation in different tissues. Many of the macrophage-related signals identified in this study have not been identified in lung tissue or peripheral blood. Future studies can further improve our understanding of methylation at the cellular level in the lung and other tissues by incorporating rapidly emerging

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single-cell sequencing methodologies. Studies that leverage bulk sequencing of complex tissues often identify molecules highly associated with changes in cell-type abundance and cannot distinguish signals within cells from different cell populations. Future studies should also include individuals of diverse ancestry and sites to further assess the effects of ancestry and environment on disease. Future studies could also benefit from analyzing DNA methylation at multiple time points. Studies have shown that baseline methylation in COPD can predict later events, but no studies have investigated changes in methylation over the disease course. Ongoing work from multiple large consortia such as COPDGene and SPIROMICS (Subpopulations and Intermediate Outcome Measures in COPD Study) to produce COPD omic data will further accelerate our understanding of the disease as the numbers of available samples, tissues, and assays continue to increase. The study by Ström and colleagues lays an excellent groundwork for future omics studies of BAL cells to capture disease-relevant signals in COPD. ■

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