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Epigenetic Changes and Immunological Features of Chronic Obstructive Pulmonary Disease

The Obstructive Lung disease in Northern Sweden (OLIN) studies
Thesis XXIV

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Akademisk avhandling

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Abstract

Background. Chronic obstructive pulmonary disease (COPD) is a heterogenous and chronic inflammatory syndrome with the lungs as its main target organ. Clinically, COPD is characterized by airflow limitation, chronic respiratory symptoms, and many extrapulmonary comorbidities. Tobacco smoke is the main environmental risk factor, but pollutants and smoke from biomass fuel are also major contributors. Why some, but not all, smokers develop the disease is a key but largely unresolved research question. Genetic factors seem to explain 40—60% of COPD susceptibility, but what additional role epigenetic factors such as DNA methylation might play has not been thoroughly investigated. Immune cells are of vital importance in the COPD pathogenesis. Among airway lymphocytes, cytotoxic CD8+ T cells are most often found to be involved in the disease, but other lymphocyte populations are not as well studied. Among patients with manifest COPD, the rate of decline in lung function differs widely. Smoking cessation decreases the rate, but beyond that, it is not well understood why some patients experience a more rapid and some a much slower disease progression. Rapid decline is associated with a poor prognosis and has been recognized as a separate phenotype of COPD. **Aim**. The overall aim of this thesis was to examine the immunologic and epigenetic features of COPD with a focus on the rapid decline phenotype, using flow cytometry and measurement of DNA methylation in cells from bronchoalveolar lavage (BAL) fluid together with clinical characteristics such as rate of decline in lung function, use of inhaled corticosteroids and smoking status. The studies included in this thesis were all part of the Respiratory and Cardiovascular Effects in COPD ("KOLIN") study. **Methods.** The study population was the same for all studies included in this thesis. Subjects were recruited from the Obstructive Lung Disease in Northern Sweden (OLIN) COPD study according to predetermined criteria. OLIN COPD also provided the longitudinal data needed for classification of rapid/non-rapid decliners (decline in forced expiratory volume in the first second [FEV1] ≥60 or ≤30 mL/year respectively). BAL fluid was analyzed for cell type composition using flow cytometry. DNA methylation in BAL cells was measured using the Illumina MethylationEPIC BeadChip. In the statistical analysis, flow cytometry data was analyzed using group-wise comparisons and multivariable regression models. DNA methylation data was analyzed for association with COPD and accelerated epigenetic aging (defined as the difference between chronological and epigenetic age) using multilinear regression models. Differentially methylated positions and regions associated with COPD were analyzed for gene association and pathway enrichment and integrated with data from previous gene expression and genome-wide association studies. **Results**. *Paper I:* in this first paper based on flow cytometry, we focused on cytotoxic lymphocytes and found that Natural Killer (NK) cells in BAL were increased in COPD while invariant Natural Killer T (iNKT) and Natural Killer T-like (NKT-like) cells increased with smoking but not with COPD. NK cells were also higher when comparing ex-smokers with and without COPD. No significant differences were found between COPD subjects with a rapid vs. a non-rapid decline in lung function. *Paper II:* regulatory immune cells were investigated in this second flow cytometry-based paper. We found that FoxP3+ regulatory T cells (Tregs) were significantly decreased in COPD subjects with a rapid decline in lung function compared to those with a non-rapid decline. This result was significant before as well as after adjustments for inhaled corticosteroids (ICS) usage and smoking. None of the investigated regulatory immune cell populations (T helper cells, activated T helper cells, and FoxP3+ Tregs) displayed significant differences associated with either COPD or smoking. *Paper III:* measurements of BAL cell DNA methylation revealed epigenome-wide differential methylation in COPD; 1,155 differentially methylated positions (DMPs) and 7,097 differentially methylated regions. Functional analysis using Kyoto Encyclopedia of Genes and Genomes and Gene Ontology databases identified biologically plausible pathways and gene relationships, including enrichment for transcription factor activity. No correlation was found between COPD and accelerated aging. For 79 unique DMPs, DNA methylation correlated significantly with gene expression in BAL. Thirty-nine percent of DMPs were co-located with single nucleotide polymorphisms (SNPs) associated with COPD. **Conclusions**. Among cytotoxic cell types, the NK cell population stood out as it 1) was increased in COPD; and 2) did not normalize in COPD subjects that had quit smoking. This indicates that NK cells might contribute to the continued disease progression in COPD even after smoking cessation. COPD subjects with a rapid decline in lung function had significantly lower levels of Fox P3+ Tregs in BAL. Further longitudinal research is needed to establish the causal direction of this relationship, but based on the evidence available to date, I deem it more plausible that a low expression of Fox P3+ Tregs would lead to a rapid decline in lung function, than the other way around. Our epigenome-wide association study (EWAS) identified widespread differential methylation in COPD, and many DMPs displayed a strong correlation with gene expression. Somewhat less than half of DMPs were located in close proximity to COPD-associated SNPs, suggesting that these might be sites where genetic factors regulate methylation status. In sum, our findings suggest strong associations between epigenetic factors and COPD. As this was the first ever published EWAS of COPD based on BAL cells, results must be validated in future studies.

Keywords

COPD, lung function decline, immunology, DNA methylation, epigenetics, OLIN, KOLIN

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