Introduction

Airflow obstruction, the hallmark characteristic of Chronic Obstructive Pulmonary Disease (COPD) has long been attributed to a combination of small airways disease and emphysema destruction.

We have previously demonstrated that gene-expression changes associated with morphological measurements of distal emphysema derived from microcomputed tomography images can provide insights into the pathogenesis of severe COPD. However, if we are to modify COPD progression it is important to understand the molecular determinants involved in the early disease (Mild/Moderate) stages. The objective of this study was to determine the genes associated with emphysema and small airways disease in mild and moderate COPD patients.

Specific Aim

To determine the underlying molecular determinants of emphysema and small airways disease in a cross sectional cohort of COPD. And subsequently identify therapeutics which may reverse patterns of pathological gene expression.

Methods & Materials

In a cross sectional COPD cohort 8 randomly sampled lung tissue cores (per 410 total samples) were scanned by microCT to determine the mean linear intercept (Lm) and number of terminal bronchioles, and 30% of tissue from each sample was used for RNA extraction and microarray analysis using the Affymetrix HuGene L.0 ST chip. Raw microarray data was transformed and quantile normalized using the robust multi array average (RMA) method. Linear model effects models (LME) and Weighted gene co-expression network analysis (WGCNA) were used to correlate gene microCT measures of emphysema and small airways disease. The Connectivity Map Cmap/Dato is a library containing over 1.5M gene expression profiles from ~1,000 small molecule compounds, and ~1,000 genetic reagents, tested in 35 cell types. Users can input gene expression signatures and find compounds which induce or reverse the query signature.

Study Design

In this study, we characterized the gene expression profiles of emphysema and small airways disease in COPD patients using microCT imaging and microarray analysis. A total of 35 COPD patients were included in the study, consisting of 19 current smokers and 16 former smokers. The disease severity of the patients was classified into mild (n=10), moderate (n=10), and severe (n=15) based on the exacerbation rate and the degree of airflow obstruction.

Patient Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n=37)</th>
<th>Gold I (n=35)</th>
<th>Gold II (n=34)</th>
<th>Gold III (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>37</td>
<td>35</td>
<td>34</td>
<td>41</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>52/2</td>
<td>67/4/0 (34)</td>
<td>62/2/13/0 (24)</td>
<td>50/17 (24)</td>
</tr>
<tr>
<td>Cigarette Pack years</td>
<td>73.2 (98.46)</td>
<td>1067.8 (58.81)</td>
<td>615 (162.73)</td>
<td>753 (302.46)</td>
</tr>
<tr>
<td>BMI</td>
<td>27.0 (3.3)</td>
<td>25.17 (3.23)</td>
<td>25.47 (3.96)</td>
<td>24.53 (3.62)</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>30.12 (3.3)</td>
<td>89.05 (3.50)</td>
<td>65.45 (3.41)</td>
<td>22.35 (3.66)</td>
</tr>
</tbody>
</table>

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Gene Expression Profiles of Emphysema and Small Airways Disease

Figure 1: using microCT we specified the amount of small airways disease and emphysema in all lung tissue cores. The percentage of diseased small airways is significantly increased in all 350 disease categories compared to control patients, increasing the levels of normal patients as measured by microCT is only significantly increased in 500 subjects, compared to all other groups.

Control patients have a lower percentage of diseased small airways compared to the diseased patients. The percentage of diseased small airways is significantly increased in all 350 disease categories compared to control patients, increasing the levels of non-diseased patients as measured by microCT is only significantly increased in 500 subjects, compared to all other groups.

Innate and Adaptive Immunity Gene Co-Expression Modules in Emphysema and Small Airways Disease

Figure 2: To the heating each column represents a sample and each row is a gene. Samples are ordered from low to high expression from left to right. Red indicates relative high gene expression and blue indicates relative low gene expression. We searched for co-expression modules, and in the first stage, the genes of each expression module changes were collected.

Overlapping pathological gene expression signatures

Figure 3: The way we used to select the overlap between all pathological gene expression signature generates the low-rank effect models.

Gene signature associated with Emphysema

Figure 4: Immune cells were reprogrammed from the lung tissue microCT data using Clue. Immune cells were then correlated against six and properties of disease transcriptional in 1 week. The microCT genes were found to significantly correlate with both emphysema and small airways disease (RMA=0).

Gene signature associated with Small Airways Disease

Figure 5: The 5 way some diagram shows the overlap between all pathological gene expression signature generated using the low-rank effect modules.

Identification of Therapeutics with the Connectivity Map

In this study, we evaluated the potential of PI3K/mTOR inhibitors to affect gene expression in COPD patients. We used the Connectivity Map to identify potential targets for the evaluation of gene expression signatures of emphysema and small airways disease.

Innate and Adaptive Immunity Gene Co-Expression Modules in Emphysema and Small Airways Disease

Figure 6: Using WGCNA we identified co-regulated gene cluster and adaptive immunity genes modules. These genes are significantly correlated with small airways disease and emphysema. The relative expression of these genes clusters increases (purple – red) in both small airways disease and emphysema.

Potential role of PI3K/mTOR Inhibitors in COPD

Figure 7: The four families of class I PI3K are shown in orange boxes. PI3Kδ, PI3Kγ, and mTOR control key aspects of inflammation and adaptive immunity including lymphocyte activation, differentiation and tolerance. Inhibitors of these pathways can induce genes involved in emphysema and small airways disease in our study.

Conclusions

Together these data suggest that:

- Small airways disease and emphysema feature common underlying molecular pathways.
- Co-expressed networks of innate and adaptive immune genes are the most highly correlated with emphysema and small airways disease.
- The Connectivity map identified PI3K and mTOR inhibitors as potential targets to reverse gene expression signatures of emphysema and small airways disease.

References


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