Combining microCT imaging and OMICs to find new therapeutics for Small Airways Disease & Emphysema in COPD

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a place of mind
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Introduction

Airflow obstruction, the hallmark characteristic of Chronic Obstructive Pulmonary Disease (COPD) has long been attributed to a combination of small airways disease and emphysematous destruction[1].

We have previously demonstrated that gene-expression changes associated with morphological measurements of end-stage emphysema derived from microCT measurements can provide insights into the pathogenesis of severe COPD[2]

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

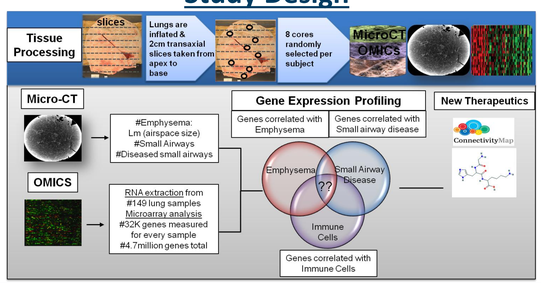
To determine the underlying molecular determinants of emphysema and small airways disease in a cross sectional cohort of COPD. And subsequently identify therapeutics which may reverse patterns of pathological gene expression.

Methods & Materials

In a cross sectional COPD cohort 8 randomly sampled lung tissue cores per case (149 total samples) were scanned by microCT to determine the mean linear intercept (Lm) and number of terminal bronchioles, and 30mg of tissue from each sample was used for RNA extraction and microarray analysis using the Affymetrix Hugene 1.0 ST chip. Raw microarray data was log2 transformed and quantile normalized using the robust multi-array average (RMA) method. Linear mixed effect models (LME) and Weighted gene co-expression network analysis (WGCNA) were used to correlate gene expression with microCT measures of emphysema and small airways disease.

The Connectivity Map Cmap/Clue.io[3] is a library containing over 1.5M gene expression profiles from ~5,000 small-molecule compounds, and ~3,000 genetic reagents, tested in 9 cell types. Users can input gene expression signatures and find compounds which induce or reverse the query signature.

Study Design



Patient Demographics

Characteristic	Control (n=5)	Gold I (n=5)	Gold II (n=5)	Gold IV (n=6)
Number of samples	37	35	36	41
Gender (M/F)	2/3	3/2	2/3	5/1
Age	59.2 (2.28)	67.4 (9.04)	60.2 (12.36)	59.17 (2.14)
Cigarette Pack years	732.2 (261.84)	1067.6 (688.1)	616 (162.73)	750 (302.46)
BMI	27.06 (3.25)	25.17 (3.23)	25.47 (5.96)	24.53 (2.62)
FEV1 %Predicted#	90.12 (8.36)	88.04 (5.93)	65.45 (8.41)	22.33 (6.68)
FEV/FVC†	73.34 (2.59)	61.75 (4.65)	59.54 (9.62)	29.5 (7.82)

Gene Expression Profiles of Emphysema and Small Airways Disease

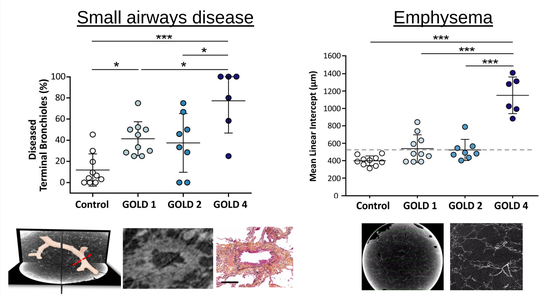
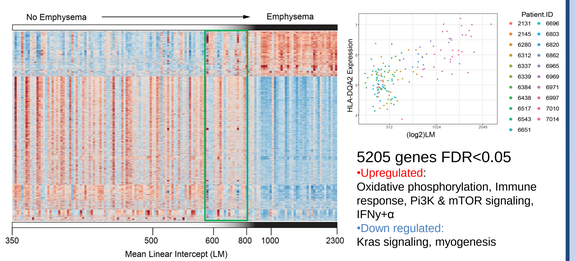


Figure 1: Using microCT we quantified the amount of small airways disease and emphysema in all lung tissue samples. The percentage of diseased small airways is significantly increased in all GOLD categories compared to control smokers. Contrastingly the level of emphysema (as measured by Lm) is only significantly increased in GOLD IV subjects, compared to all other groups.
*** denotes P<0.05, ** P<0.01, *** P<0.001**

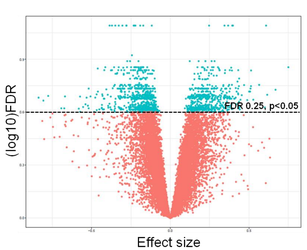
Gene signature associated with Emphysema



5205 genes FDR<0.05
•Upregulated:
Oxidative phosphorylation, Immune response, Pi3K & mTOR signaling, IFNγ+α
•Down regulated:
Kras signaling, myogenesis

Figure 2: In the heatmap each column represents a sample and each row is a gene. Samples are ordered from low to high emphysema from left to right. Red indicates relative high gene expression and blue indicates relative low gene expression. At Lm >600 the pattern of gene expression begins to change Genes which are upregulated with increasing emphysema are involved in inflammation, oxidative phosphorylation, and Pi3K/mTOR signaling. Genes which are downregulated with increasing emphysema are involved in Kras signaling and myogenesis.

Gene signature associated with Small Airways Disease



1338 genes FDR 0.25, p<0.05
•Upregulated:
Oxidative phosphorylation, Reactive oxygen species, Immune response, Pi3K & mTOR signaling
•Down regulated:
Mitotic spindle, Microtubule & Cilium morphogenesis

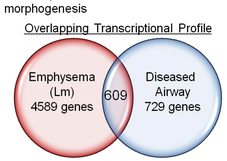


Figure 3: Volcano plot shows all genes and those significantly correlated with number of Diseased Terminal Bronchioles using the linear mixed effect model (FDR<0.25). Genes which are upregulated with increasing diseased bronchioles are involved in oxidative phosphorylation, inflammation, and Pi3K/mTOR signaling, and EMT. Genes which are downregulated with increasing diseased bronchioles are involved in mitosis, microtubule and cilium movement and morphogenesis.

Innate and Adaptive Immunity Gene Networks associate with in Emphysema and Small Airways Disease

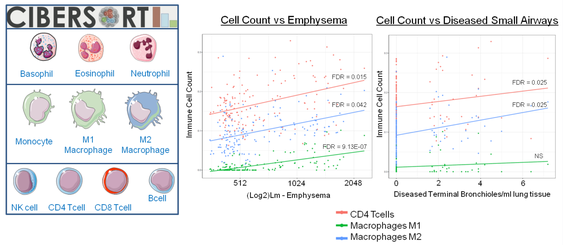
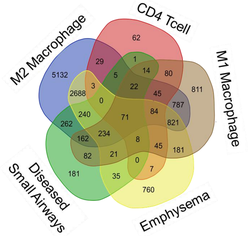


Figure 4: Immune cell counts were enumerated from the lung tissue microarray data using Cibersort. Immune cell counts were then correlated against Lm and proportion of diseased terminal bronchioles in a linear mixed effect model. CD4 Tcells and macrophages were found to significantly correlate with both emphysema and small airways disease (FDR<0.05).

Overlap of pathological gene expression signatures



71 genes are shared by all signatures
But…
>5000 are shared by at least 2 signatures
- Could genes be acting in networks???

Figure 5: The 5 way venn diagram shows the overlap between all pathological gene expression signatures generated using the linear mixed effect models.

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

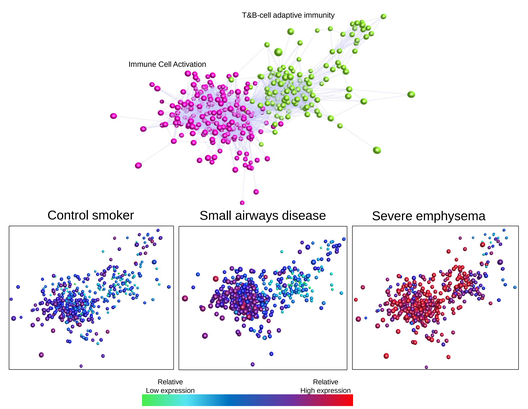


Figure 6: Using WGCNA we identified co-expressed gene Innate and adaptive immune genes modules, which are significantly correlated with small airways disease and emphysema. The relative expression of these gene clusters increases (purple – red) in both small airways disease and emphysema.

Identifying Therapeutics with the Connectivity Map

In 8 out of 9 cell lines Pi3K & mTOR inhibitors reverse both:
•The Emphysema signature (-93.58)
•The Small airways disease signature (-99.40)

Figure 7: The circus plot shows the transcriptional activity of all 9 cell lines when treated with Pi3K inhibitor PI-103. Robust transcriptional activity is observed across 8 cell lines, indicated by the connecting lines.

Pi3K Inhibitor Pi-103 reverses Emphysema and Small airways disease signatures in A549 Cells

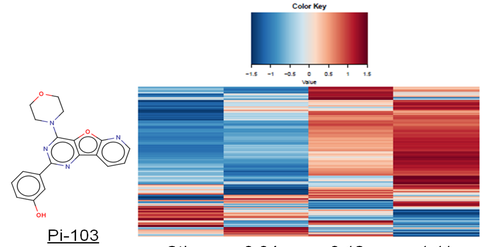


Figure 8: Treatment of A549 cells reverses expression of genes significantly correlated with Emphysema and small airways disease in a dose dependent manner. i.e. Blue genes in Control (Ctl) become Red, and Red genes in Ctl become Blue

Conclusions

Together these data suggest that:

- Small airways disease and emphysema feature common underlying molecular pathways
- Co-expressed networks of Innate and adaptive immunity 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

Potential role of Pi3K/mTOR Inhibitors in COPD

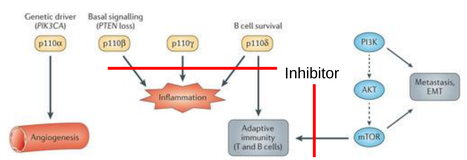


Figure 9: The four isoforms of class I PI3K are shown in orange boxes. p110β,γ,δ, and mTOR control key aspects of inflammation and adaptive immunity, including lymphocyte activation, differentiation and tolerance. Inhibition of these pathways can reverse genes involved in emphysema and small airways disease in our study.

References

1. Hogg, J. C. (2004). Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *The Lancet*, *364*(9435), 709-721.
2. Campbell, J. D., McDonough, J. E., Zeskind, J. E., Hackett, T. L., Pechkovsky, D. V., Brandsma, C. A., ... & Xiao, J. (2012). A gene expression signature of emphysema-related lung destruction and its reversal by the tripeptide GHK. *Genome medicine*, *4*(8), 67.
3. https://clue.io/
4. Fruman, D. A., & Rommel, C. (2014). PI3K and cancer: lessons, challenges and opportunities. *Nature reviews Drug discovery*, *13*(2), 140-156.