Evaluating Clinical Relevance of Animal Models In Chronic Obstructive Pulmonary Disease (COPD) Through Transcriptomic Changes in Humans and Mice

Anjali Kumari[1,2], K. Monica Lee[1], Sreepriya Pramod[1], Chastain Anderson[1], Jesse Fredrick[1], Lionel Deloach[1], and Alex Jordan[1]

[1]Altria Client Services LLC, Richmond, VA; [2]North Carolina Agricultural and Technical State University

Project Outline

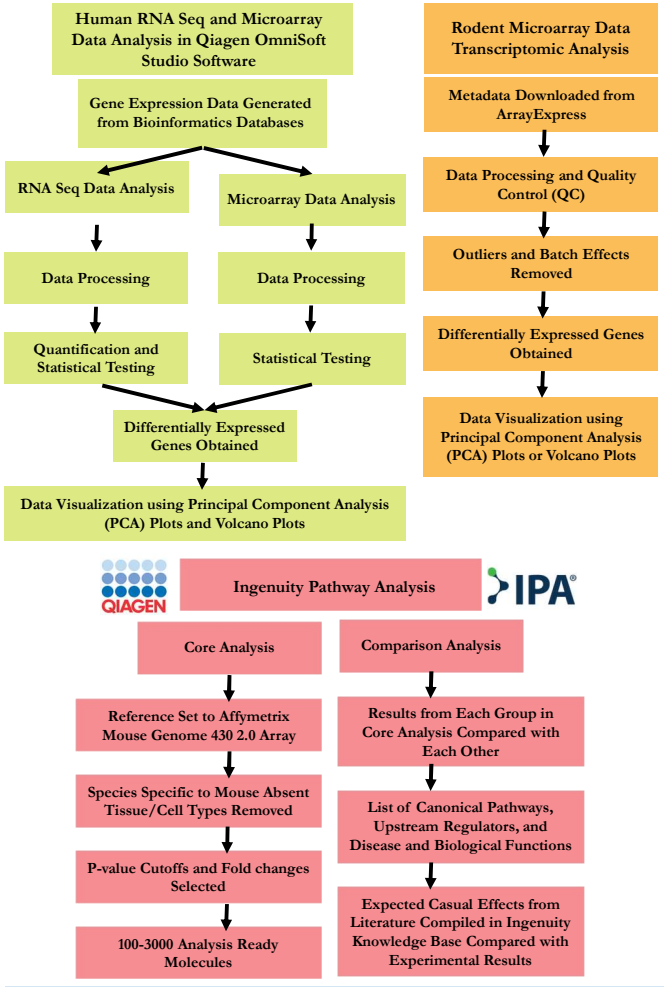
Background and Purpose: Cigarette smoking causes chronic diseases including lung cancer, cardiovascular disease, and chronic obstructive pulmonary disease (COPD). While cessation is the most effective approach to minimize smoking-related disease, novel smoke-free tobacco or nicotine products such as e-vapor or heat-not-burn products offer potentially reduced-risk (PRR) alternatives to smokers unwilling to quit. PRR products typically contain significantly lower levels of smoke-related toxicants, yet their long-term risk is unknown with limited human data. However, evidence for PRR has been indirectly shown using nonclinical (animal) models of chronic inhalation.

Methods: Animal models provide a holistic in vivo system that shares many physiological, anatomical, and genetic resemblances with humans. In this study, we have utilized publicly available gene expression data from COPD mouse models and compared their gene expression (microarray) changes against existing human COPD data (QIAGEN HumanDisease_B38_GC33 land; QIAGEN, Redwood City, CA). Three objectives include: 1) analyzing human microarray data, 2) analyzing mouse microarray data, and 3) conducting pathway analysis to identify COPD-related canonical pathways, upstream regulators, and biological functions from both datasets. The evaluated human data include differentially expressed genes in healthy smokers, healthy non-smokers, smokers with COPD, and non-smokers with COPD. The data also includes transcriptomic changes during transition from healthy smoker to COPD smoker and from early to late COPD. Mouse data were evaluated to identify pathways impacted during early (1-month) and chronic (up to 7-month) cigarette smoke exposure and any differential pathways activated with PRR product exposures, as well as differences in pathway regulation due to cessation or switching from cigarette to PRR products. Finally, Ingenuity Pathway Analysis (IPA) was used to compare significant upstream regulators and pathways within and between human and mouse data.

Results & Discussion: Despite differences in experimental methods and environmental conditions in animal COPD models, the results demonstrate qualitative similarities in the increase of immune cells and significant up and down-regulation of neuronal activity that is associated with cigarette smoke-related COPD progression in humans. At the same time, the context for interpreting and applying the outcomes from secondary analyses needs to be defined and the caution in predicting human outcomes from animal models are warranted.

In conclusion, with defined experimental workflows in analyzing different study outcomes, animal models can be valuable and informative to investigate potential long-term clinical outcomes.

Methods



References

Mouse Models of smoking-related COPD:

1. Kumar et al (2021). A 7-month inhalation toxicology study in C57BL/6 mice demonstrates reduced pulmonary inflammation and emphysematous changes following smoking cessation or switching to e-vapor products. *Toxicology Research and Application, 5*.
2. Lee et al (2018). Biological changes in C57BL/6 mice following 3 weeks of inhalation exposure to cigarette smoke or e-vapor aerosols. *Inhal Toxicol, 30*(13-14), 553-567.
3. Phillips et al (2015). A 7-month cigarette smoke inhalation study in C57BL/6 mice demonstrates reduced lung inflammation and emphysema following smoking cessation or aerosol exposure from a prototypic modified risk tobacco product. *Food Chem Toxicol, 80*, 328-345.

Human Data on COPD:

1. Qiu, F., Liang, C. L., ... & Dai, Z. (2017). Impacts of cigarette smoking on immune responsiveness: Up and down or upside down?. *Oncotarget, 8*(1), 268.
2. Raman, T., O'Connor, T. P., ... Crystal, R. G. (2009). Quality control in microarray assessment of gene expression in human airway epithelium. *BMC Genomics, 10*, 493.

Results

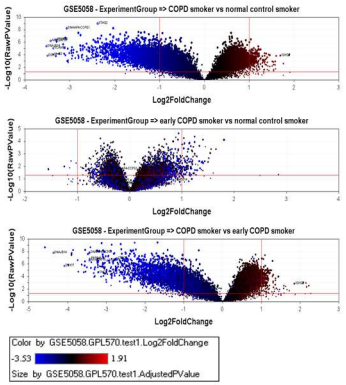


Figure 1: Transcriptomic changes in small airway epithelium (from human data)
Number of differentially expressed genes increase with progressing stages of COPD

Limitation: The results are considered preliminary based on limited sample size and number of clinical studies, specific lung tissue types used for analysis, differences in type of PRR products, and environmental factors affecting humans.

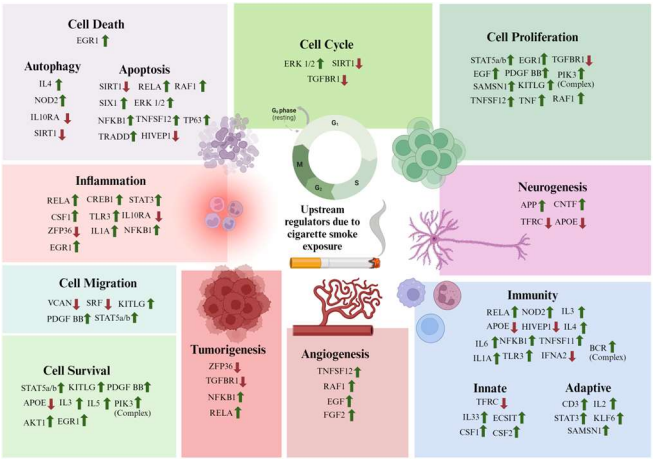
Upstream Regulators	3month_3R4F	4month_3R4F	7month_3R4F	COPD_GOLD_I	COPD_GOLD_IV
AKT1	2.344	1.577	3.09	0.416	2.554
APOE	-4.643	-4.473	-5.182	-1.624	-2.029
APP	4.229	4.264	4.637	1.467	2.268
BCR (complex)	2.032	2.441	2.162	1.699	3.251
CD3	3.267	3.153	3.484	1.532	2.538
CNTF	4.427	4.494	4.667	2.331	2.956
CREB1	2.207	1.584	2.228	2.177	2.556
CSF1	4.301	4.191	4.855	0.364	2.187
CSF2	5.274	4.942	7.304	1.819	3.227
ECSIT	1.941	2.393	2.177	2.708	3.302
EGF	3.276	2.419	4.803	0.967	4.227
EGR1	3.906	3.363	4.112	0.452	2.233
ERK	2.141	1.14	2.554	0.774	2.584
ERK1/2	3.102	2.867	3.122	1.814	3.453
FGF2	2.362	1.784	2.54	0.251	2.686
HGF	1.385	2.029	2.356	2.442	2.644
HIVEP1	-2.828	-3.317	-3.317	-1.915	-2.517
IFNA2	2.421	2.279	2.302	4.096	0.915
IL10RA	-4.71	-4.666	-5.834	-3.138	-4.139
IL1A	5.02	5.106	5.085	1.742	3.714
IL2	4.855	4.883	4.89	1.815	2.288
IL3	2.232	2.897	3.63	0.919	2.551
IL33	4.714	5.005	5.362	1.233	2.232
IL4	5.006	4.988	5.252	2.194	3.252
IL5	3.608	3.622	4.608	1.382	3.561
IL6	3.9	4.317	4.661	1.823	4.371
KITLG	2.287	2.325	2.921	1.919	2.256
KLF6	4.362	4.273	5.069	2.195	3.906
NFKB1	3.615	2.794	4.402	1.278	2.678
NOD2	3.409	3.414	3.545	2.224	2.782
PDGF BB	2.674	2.478	4.238	3.456	3.872
PI3K (complex)	4.048	4.046	4.682	0	2.131
RAF1	2.596	2.416	2.814	1.34	3.026

Table 1: Examples of Common Upstream Regulator Trends in Rodent and Human Model

- In Table 1, majority of upstream regulators for both human and mouse models regulate immune responses (cell movement of immune cells, phagocytosis, adhesion of immune cells, etc.) or tumorigenesis pathways (growth of tumor, growth of lesion, metastasis, extra-pancreatic malignant tumor)
- Studies also demonstrate significant up and down regulation neuronal/ CNS-related activity

Figure 3: Upstream regulators that demonstrating similar relative trends between human and mouse models using Qiagen IPA analysis following cigarette smoke exposure

- Figure 3 provides overall trends in biomolecular changes during the onset of COPD (data from Qiagen IPA analysis), supporting similarities in relative regulator expression trend when comparing mouse and human as screening models for PRR product development relative to cigarettes.



Acknowledgements: Qiagen Digital Insights team (US & Romania); Ivana Grbesa, and NC A&T SU