Is electronic cigarette inhalation toxic for healthy never smokers? Bioinformatics reveals the answer

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OBJECTIVES

The use of electronic cigarettes (EC) is rapidly gaining popularity. It is estimated that 8 to 10% of US smokers have used EC, and there is increasing initiation of EC smoking in younger age groups with no prior smoking history (1). EC are marketed as a substitute for cigarettes that deliver nicotine but not the toxic products of cigarette smoke, and are used as a strategy to reduce cigarette smoking, with the concept that EC are safer.

The aim of the study was to evaluate the consequences to the biology of the human lung of healthy never smokers following acute exposure to EC aerosols and to investigate the potential toxicities induced by EC inhalation.

For this purpose we analyzed gene expression data (GSE85121) from small airway epithelium (the first site of lung abnormalities in cigarette smokers) and alveolar macrophages (the mononuclear phagocyte defenders of the lower respiratory tract) of 5 healthy never smokers following inhalation of total 20 puffs of "Blu" brand EC with nicotine (1).

METHODS

We used our proprietary database and software for data interpretation and visualization. The database was generated to include internally curated and various publicly available data on proteins, biologically-active chemicals, their interactions, pathways and pathologies (Figure 1). Four million references were catalogued, supporting each database entry, with hyperlinked interactions to appropriate PubMed articles as support, whereas proteins and chemicals were hyperlinked to EntrezProtein and PubChem, respectively. In order to support data integration and capture different levels of histopathological observations, we developed toxicology ontology with over 2500 toxicity endpoints.

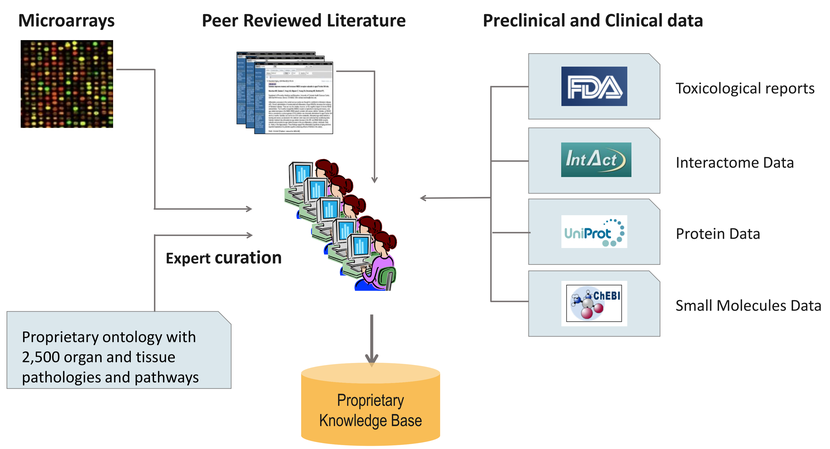

Figure 1. Manually curated database. Data was extracted from many diverse sources. Database contains complete human interactome, 2,500 organ and tissue pathologies, 500,000 synonyms/ontologies, 4 million linked articles (PubMed, FDA, clinical, patents), >10 million relationships, 50,000 biologically active chemicals.

RESULTS

From the whole genome analysis, using the cut off criteria of $p<0.05$ and $FC>\pm1.5$, differentially expressed genes (DEGs) were identified in the small airway epithelium and in the alveolar macrophages (Table 1). Mapping dysregulated genes to Gene function cluster within our system revealed that in small airway epithelium and alveolar macrophages, EC inhalation influenced genes associated with numerous biological processes (Table 2). In order to find potential respiratory system toxicities following EC inhalation, we mapped dysregulated genes to Disease and Organ and tissue pathologies clusters within our system (Table 3).

Table 1. Number of differentially expressed genes (DEGs) upon exposure to electronic cigarettes.

	Small airway epithelium	Alveolar macrophages
Total number of DEGs	97	92
Total number of DEGs recognized by our system	72	60
Up-regulated	11	41
Down-regulated	61	19

Table 2. A sample of GO biological processes (BPs) associated with DEGs following EC inhalation. BPs associated with genes induced upon EC exposure are in red boxes and BPs associated with downregulated genes are in green boxes.

	GO biological processes	
Small airway epithelium	Anti-apoptosis, wound healing-spreading of epidermal cells, angiogenesis	Inflammatory, immune and cellular defense responses
Alveolar macrophages	Collagen catabolic process, DNA replication, response to DNA damage stimulus	Cytokine activity and cell proliferation

Table 3. A sample of diseases and pathologies following EC inhalation in the respiratory system.*Dataset from small airway epithelium, ** dataset from alveolar macrophages. Molecules inside are directly involved in the pathology, molecules next to are "guilty by association".

Respiratory system pathologies	Molecules inside	Molecules next to
Asthma*	NCF4, FKBP5, SPI1, S100A8, CX3CR1, PLAU, CXCL9, CCR5, CD36, HMOX1, PDE4B	MKI67, CD163, S100A9, TOP2A, THBS1…
Interstitial lung disease*	CD163, S100A9, PLAU, CCR5, HMOX1	TOP2A, CD36, MKI67, PDE4B…
Acute respiratory distress syndrome*	S100A8, S100A9, PLAU, HMOX1	CD36, CCR5, TOP2A, MKI67
Lung inflammation*	S100A8, S100A9, CX3CR1, PLAU, CXCL9, CCR5, CD36, HMOX1	CD163, TOP2A, MKI67, SPI1…
Lung fibrosis*	S100A8, S100A9, PLAU, CD36, HMOX1	CCR5, TOP2A, MKI67, CXCL9…
Alveolar macrophage hypertrophy**	MMP2, MMP9	MMP7, AREG, ALDH7A1, RRM2…

Vast amount of processed data in our system presents the potential for generation of valuable mechanistic hypothesis (Figure 2). Detailed interrogation of the asthma network combined with differentially expressed genes from the experimental datasets, using our proprietary database, revealed EC inhalation let to suppression of the genes involved in inflammatory response.

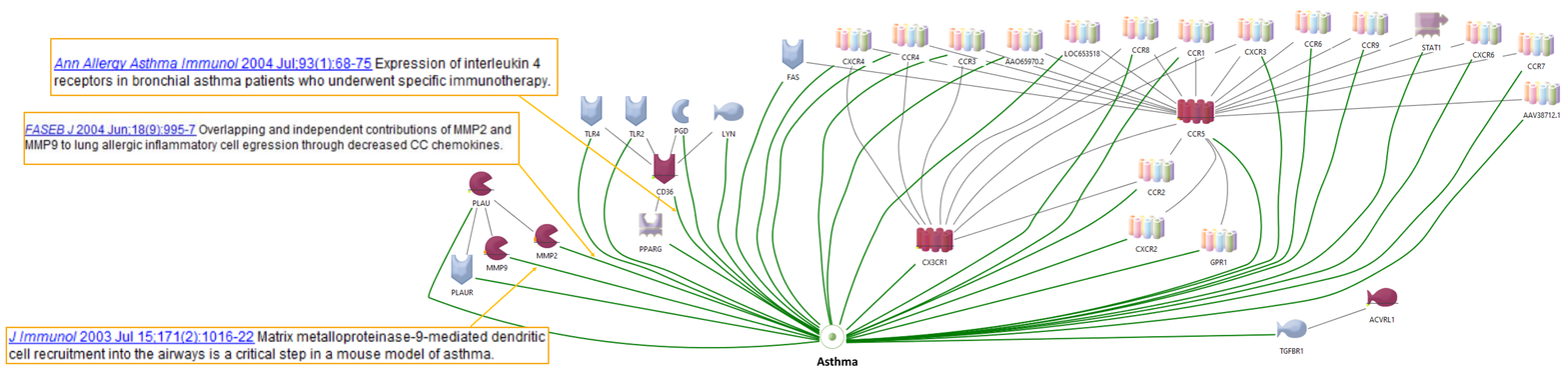

Figure 2. A segment of asthma pathway analysis. Proteins in purple are differentially regulated upon EC inhalation. Clicking on the connector lines within network provides easy access to literature evidences enabling postulating hypothesis.

CONCLUSION

- We have shown that acute inhalation of EC aerosols dysregulates normal human lung homeostasis in a limited cohort of healthy naïve individuals.
- We have identified several potential respiratory system toxicities associated with EC inhalation. We generated the mechanistic hypothesis showing that EC may dysregulate the genes in pre-existing conditions such as asthma.

REFERENCES

1. Staudt MR *et al.*, Respir Res. **2018** May 14;19(1):78.