Organotypic tissue cultures as models to study the impact of cigarette smoke on human upper and lower respiratory tract

413

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Introduction

Cigarette smoke (CS) is etiologically linked to fatal respiratory diseases. To enable the development of modified risk tobacco products (MRTPs) to reduce the risk of smoking related diseases, there is a need to understand the mechanisms whereby CS causes disease. As an alternative to animal experimentation, the principles of 3 R's necessitate the establishment of reliable in vitro models, which may also provide a better predictability of exposure response, when derived from human cells. Among the in vitro models, the three-dimensional organotypic pseudo-stratified culture systems better mimic the morphological, physiological, and molecular attributes of human respiratory tract than primary or immortalized cells cultured as a submerged monolayer.

The bronchus and lung parenchyma are the primary sites that manifest smoking related respiratory diseases, however, nasal epithelium has been proposed as a surrogate tissue to study smoking effects on the respiratory tract (1,2).

Previously, we showed that the transcriptomic responses were very similar in CS-exposed bronchial and nasal organotypic tissue cultures when analyzed in the context of a network model representing xenobiotic metabolism. In addition, the perturbation of the xenobiotic metabolism network was similar in organotypic in vitro cultures and nasal and bronchial brushings from smokers (3).

Here, we further characterize the response of nasal and bronchial organotypic cultures to whole mainstream CS.

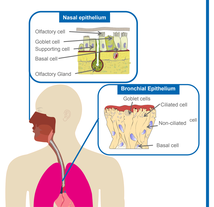

Figure 1. Organotypic tissue cultures were derived from human primary nasal and bronchial epithelial cells

Exposure Design and Quality Controls

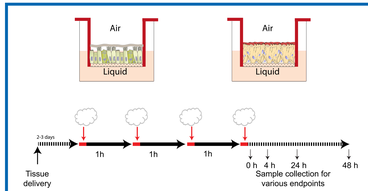

Figure 2. Organotypic nasal and bronchial tissue cultures were exposed repeatedly to mainstream CS (3R4F) at air-liquid interface.

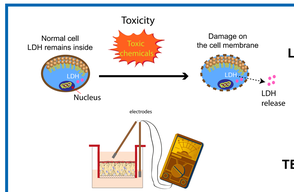

Figure 3. Cytotoxicity and tissue integrity were assessed by measuring lactate dehydrogenase (LDH) release and transepithelial electrical resistance (TEER), respectively.

References

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Results

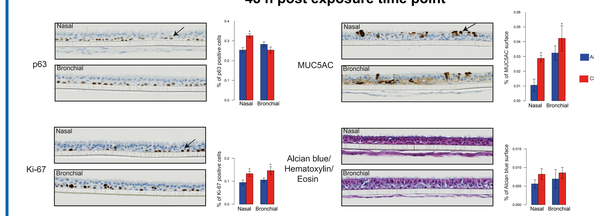


Figure 4. Representative sections of nasal (top) and bronchial (bottom) cultures 48 h after the last CS exposure. Arrows indicate the positively stained cells. The bar graphs show the quantification of the staining as a percentage (median ± standard error of the median) of all nuclei in the tissue sections for p63 and Ki-67 (percentage of stained nuclei), and the area occupied by positive cells for MUC5AC and Alcian blue (percentage of stained area). * indicates a statistically significant difference between the median of the CS and air exposed cultures for the same tissue. The Wilcoxon rank-sum test calculated the significance level as 5%.

CS exposure resulted in a small increase in the numbers of basal and proliferating cells.
The impact of CS on the Muc5Ac positive cells was more pronounced in the nasal cultures.

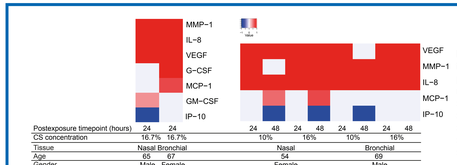


Figure 5. Heatmaps of the fold changes of inflammatory markers from two independent studies. Columns represent different post-exposure time points, CS concentrations, tissues, and donor characteristics as labeled under the heatmap. Fold changes were obtained by taking the log2 of the cytokine abundance ratio between the CS and sham exposure group for every tissue. Welch's t-test was performed to test the null hypothesis that cytokine abundance in CS and sham exposure groups are the same. Fold change was set at zero for p-values > 0.05. Blue and red indicate statistically significant negative and positive fold changes, respectively.

CS exposure resulted in the reproducible accumulation of MMP-1, IL-8, and VEGF in the culture medium of both nasal and bronchial tissue cultures and attenuated the secretion of interferon gamma-induced protein (IP-10) by nasal cultures.

The accumulation of IL-10, IL-1A, IL-1B, IL-6, MIP-1A, MMP-9, eotaxin, and RANTES in the culture medium was not impacted (not shown) by CS exposure and the impact of CS on the secretion of MCP-1 G-CSF, and GM-CSF was not reproducible at this post-exposure time point.

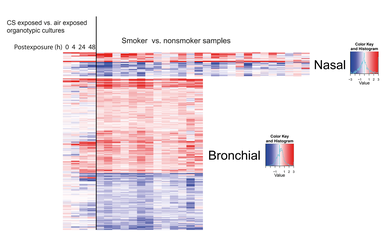


Figure 6. Comparison of cigarette smoke exposure response in in-vivo (GSE16008) (2) and in-vitro nasal and bronchial tissue cultures. Top panel: Grey and black bars denote sets of significantly up- and downregulated genes, respectively, that were significantly enriched in in-vitro organotypic bronchial tissue. NES: normalized enrichment score. ***False discovery rate (FDR) 0.001; **FDR 0.01; *FDR 0.05. Bottom panel: Gene expression fold changes in the in-vitro organotypic tissue cultures exposed to CS compared with air-exposed cultures are plotted side by side with gene expression fold changes from the in vivo study (GSE16008) obtained by taking the differentially expressed genes in smokers' samples compared to the average expression level from nonsmokers. Blue and red colors indicate negative and positive fold changes respectively.

Several differentially expressed genes in human smoker nasal and bronchial epithelial cells were also regulated in the organotypic cultures exposed to whole smoke.

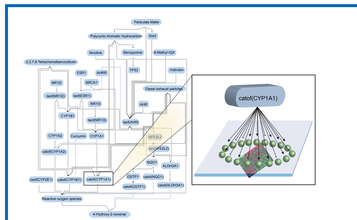
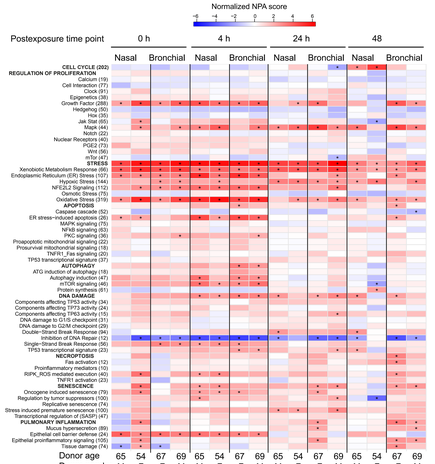


Figure 7. A biological network model (above) comprises the functional layer of the network, which is connected by causal edges carrying the directional information. The blue ovals represent the activity of the backbone nodes (i.e., the functional layer) and the green balls represent the expression of genes (i.e., the transcriptional layer) (4-8).

Network scoring assesses the impact of treatment on specific backbone entities based on the differential gene expression of downstream genes that are known to be regulated by these mechanisms (9-11).The quantitative measure of this impact is called the Network Perturbation Amplitude (NPA).

The heatmap (right) shows the perturbation of the biological networks at each post exposure time point in nasal and bronchial organotypic tissue cultures in response to CS exposure. The biological NETWORKS and sub networks are labelled on the left with the number of nodes in each network. Shown are the results from two independent experiments conducted on the tissue cultures from different donors (donor characteristics shown on the bottom). Normalized NPA score takes into account both the magnitude of network perturbation and the Specificity statistics (asterisk).



The analysis of transcriptomic data in the context of biological network models showed that whole CS exposure had a clear impact on biological processes in both nasal and bronchial cultures:

1. The cellular stress response clearly dominated in both tissue cultures throughout the post exposure period.
2. Processes related to proliferation, cell fate, and inflammation were activated and DNA repair was inhibited in both nasal and bronchial tissues in response to CS exposure.
3. While cell cycle became activated in the nasal cultures at 48 hours after exposure, bronchial cultures reproducibly showed the activation of epithelial proinflammatory signaling at this post exposure time point.

Conclusions

Both nasal and bronchial organotypic cultures showed changes in inflammatory mediator release in response to repeated exposure to mainstream CS.

While the LDH release, TEER assay, and histology analysis did not indicate cytotoxicity in response to whole smoke exposure, the transcriptomic analysis revealed the activation of biological processes related to cellular stress, proliferation, inflammation, and cell fate.

Gene expression patterns in CS exposed organotypic tissue cultures closely resembled those observed in smoker nasal and bronchial epithelium.

The state-of-the art organotypic nasal and bronchial tissue cultures are a promising system to study inhalation toxicology; they provide an alternative to animal experimentation and cells grown on a monolayer.