Systems toxicology approach for the biological impact assessment of conventional cigarette smoke fractions and aerosol fractions from a prototypic modified risk tobacco product on normal human bronchial epithelial cells

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Introduction

Smoking causes serious fatal diseases such as lung cancer and chronic obstructive pulmonary disease. The best way for smokers to reduce the adverse health effects is to quit smoking. However, there has been a growing interest in recent years in harm reduction, stimulated perhaps by the observations that in spite of the significant efforts directed towards tobacco control and communication of the risks of smoking, many smokers still have little interest and/or success in quitting smoking[1]. A significant development in tobacco control in the US has been the enactment of the Family Smoking Prevention and Tobacco Control Act (FSPTCA)[2], which empowers the US Food and Drug Administration (FDA) to evaluate and regulate Modified Risk Tobacco Products (MRTPs). The FSPTCA defines a MRTP as 'any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products.

Although the causal relationship between smoking and several diseases is well established[3], there is still little understanding of the underlying mechanisms.

With the aim to demonstrate the applicability of a systems toxicology approach to contribute to the evaluation of the risk associated with a prototypic(p)MRTP aerosol and to get mechanistic insights in key cellular processes, we have exposed normal human bronchial epithelial (NHBE) cells to an aqueous cigarette smoke fraction (smoke-bubbled phosphate-buffered saline/sbPBS) generated from the 3R4F reference cigarette and an prototypic(p)MRTP aerosol. In addition, a static headspace GC-MS method focusing on volatile and semi-volatile constituents was used to compare the amount of chemical constituents between 3R4F and pMRTP.

Methods & Study Design

NHBE cells were exposed to an aqueous cigarette smoke fraction (sbPBS) from the 3R4F reference cigarette at three non-toxic concentrations based on cell viability for either 4 or 24 hours. Exposure to pMRTP matched to the highest concentrations of 3R4F in terms of puff/ml.

Cell viability was determined after 24 hours of exposure using a resazurin assay. RNA was isolated and further processed on GeneChip Human Genome U133 plus 2.0 arrays (Affymetrix). To place the gene expression data into the context of known biology, a novel computational-modeling approach[4] based on tissue-specific causal biological networks[5-9] was applied. The computable biological network models are specific to non-diseased pulmonary and cardiovascular cells/tissues and capture the molecular events that can be activated following exposure to environmental toxicants. The biological mechanisms covered by our networks encompass cell proliferation, cellular stress, lung inflammation, DNA damage, autophagy, cell death and senescence (DACS). Each network is built in a modular way where each module (sub-network) describes a specific biological aspect of the entire network. Gene expression fold-changes were translated into differential values for each node within the network. The node differential values are in turn summarized into a quantitative measure[10].

85 volatile and semi-volatile compounds apparent in cigarette smoke and measurable by headspace conditions have been monitored and semi-quantified in sbPBS by headspace-GC-HR-MS. A heatmap was built by using the R package gplots.

References

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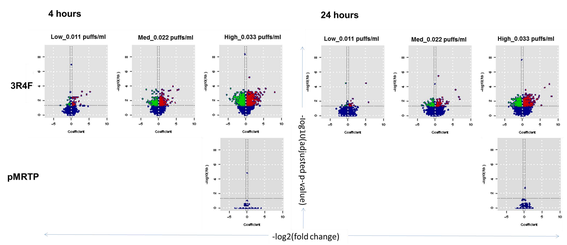
Results

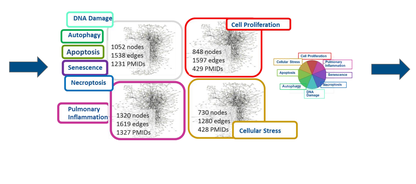
From Gene Expression Changes to Impacted Biological Processes

Systems Response Profiles of Gene Expression Data (SRP)



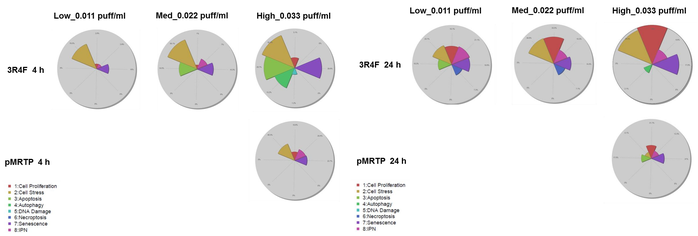
SRP demonstrate a concentration-dependent up-regulation of differentially expressed genes after 3R4F exposure. In contrast, exposure to the pMRTP led to no significant changes in the gene expression profile as compared to buffer-exposed control cells.

Biological Processes in Healthy Systems



Using a computational approach that is based on causal models of tissue-specific biological networks[4-9], gene fold-changes were translated into biological impact scores, a quantitative measure for the amount of the impact that one network undergoes when exposed to an external stimuli[7].

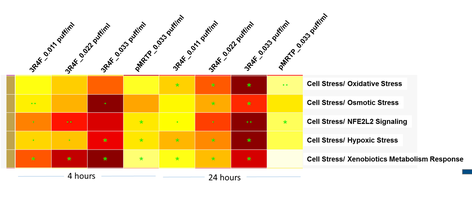
Impact on Biological Processes



The biological network analysis revealed, that most impacted networks were related to cell stress and senescence after 4 hours of exposure to sbPBS from 3R4F, whereas after 24 hours of exposure the cell proliferation network was most impacted. Exposure to the pMRTP resulted in lower perturbation of biological networks. Although no genes appeared to be significantly changed in the SRP the threshold-free network approach can show some biological impact resulting from these changes. The cell stress network shown as an example.

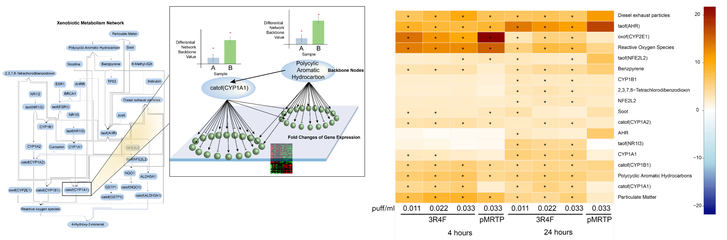
Mechanism of Impacted Biological Processes

Heatmap of Cell stress Sub-networks



The cell stress network consists of 5 sub-networks: Oxidative Stress, Osmotic Stress, NFE2L2 Signaling, Hypoxic Stress, and Xenobiotics Metabolism Response. The xenobiotics metabolism was found to be substantially impacted in a dose related manner. impacted by 3R4F exposure with a stronger response at the 4 hour time points. pMRTP exposure impacted the xenobiotics response to a lesser extent.

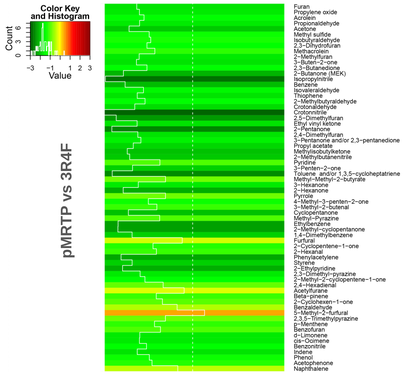
Backbone Xenobiotics Metabolism Response



The model represents the mechanism of xenobiotic metabolism and illustrates the quantification approach[11]. The activation of the nodes in the network model were predicted from the gene expression levels calculated from contrast of between the control and exposed samples. The highly contributing nodes to the overall impact on xenobiotic metabolism (i.e. the leading nodes) are displayed as a heatmap. Diesel exhaust particles, the transcriptional activity for the arylhydrocarbon receptor, the oxidation of CYP2E1 and reactive oxidative species are predicted to have a major impact on the xenobiotics metabolism perturbation. Importantly, the sum of the overall impact is lower after pMRTP exposure than after 3R4F exposure.

Smoke Chemistry of sbPBS Fractions

Heatmap of Volatile and Semi-volatile constituents



The heatmap shows the smoke chemistry monitored by headspace-GC-HR-MS of sbPBS fractions from 3R4F and pMRTP. The semi-quantitative data were transformed by log10, followed by a normalization relative to 3R4F. The value in each cell is shown by a vertical solid white line whose distance from the center of this cell (dashed white line) is proportional to the value in this cell. A histogram shows the color key.

Most of the levels of the 85 monitored volatile and semi-volatile chemicals were lower in sbPBS of the pMRTP as compared to the 3R4F.

Conclusion

In summary, we provide mechanistic insight into the biological impact on key cellular processes in NHBE cells upon sbPBS exposure, suggesting reduced biological network perturbation from exposure to pMRTP compared to 3R4F. The lower amount of chemicals detected in the pMRTP could be associated with the lower biological impact in NHBEs. In addition, we demonstrated the applicability of a systems toxicology approach to contribute to the evaluation of the risk associated with pMRTPs.

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