Comparison of the biological impact of cigarette smoke and a prototypic modified risk tobacco product on human versus rat primary normal bronchial epithelial cells

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Introduction

Smoking causes serious diseases such as lung cancer, cardiovascular and chronic obstructive lung diseases. Undoubtedly, the best way for smokers to prevent the adverse health effects of tobacco is to quit smoking. For those unable or unwilling to quit smoking, growing attention in alternative approaches including that of harm reduction emerged in recent years[1]. For example, the US Family Smoking Prevention and Tobacco Control Act (FSPTCA) empowers the US Food and Drug Administration (FDA) to evaluate and regulate Modified Risk Tobacco Products (MRTPs)[2]. MRTP means 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. Consistent with the current global paradigm change for safety assessment of consumer products, Philip Morris International R&D is working towards creating an integrative, systems toxicology based approach for the risk assessment of MRTPs.

Inhere, the systems toxicology based approach was used to (i) compare the biological impact of a prototypic (p)MRTP to the impact of the reference cigarette 3R4F and (ii) the investigate the level of translatability between rodent and human biology. The evaluation of the biological translatability across species and between *in vivo / in vitro* systems is a corner stone in the quest of alternative approach to the animal testing suggested by the ”3R” concept (“Reduce”, “Refine”, “Replace”). Bronchial epithelial cells represent a barrier and the first line of defense against inhaled toxicants. Therefore, we exposed normal human bronchial epithelial cells (NHBE) and its rat counterpart normal rat bronchial epithelial cells (NRBE) to total particulate matter (TPM) generated from the pMRTP and from the 3R4F and measured the gene expression using whole genome chips. For analyses computational tools were applied.

Methods & Study Design

NHBE cells, purchased from Lonza Inc. (Switzerland) were derived from tracheo/bronchial epithelial tissue of a 60 years old male donor, without smoking history. NRBE cells (from CHI Scientific Inc., USA) were isolated from pooled tracheobronchial tissue of adult inbred AGA rats.

NHBE and NRBE cells were exposed in parallel to an ethanolic solution of TPM generated from mainstream smoke of the 3R4F reference cigarette or the pMRTP for 4 hours. Exposure concentration were matched between 3R4F and pMRTP by using equal puffs/L. Non-toxic concentrations were used based on the determination of cell viability after 24 hours of exposure using a resazurin assay.

Total RNA samples from NHBE and NRBE cells were hybridized on GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix, 54’675 probesets) and GeneChip Rat Genome 203 2.0 Array (Affymetrix, 31’099 probesets), respectively.

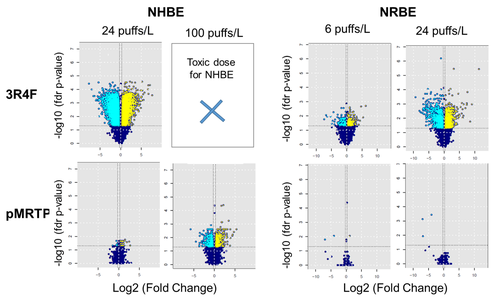
To analyze and interpret the gene expression data in the context of known biology, a novel computational-modeling approach[4] based on tissue-specific causal biological networks[5-8] was applied. The computable biological network models are specific to non-diseased pulmonary and cardiovascular cells/tissues and capture the molecular events that can be activated following exposure to environmental toxicants. The biological mechanisms covered by these networks encompass cell proliferation, cellular stress, lung inflammation, DNA damage, autophagy, cell death (apotosis, necroptosis) and senescence. 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 were in turn summarized into a quantitative measure that reflects the overall network perturbation[9].

For translational approach, networks that were significantly perturbed in both species were used to compute the correlation coefficient (Pearson) between the differential network nodes values from rat and human networks.

Results

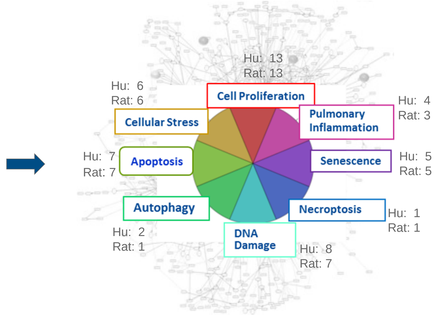
From Gene Expression Changes to Impacted Biological Processes

Systems response profiles of gene expression data



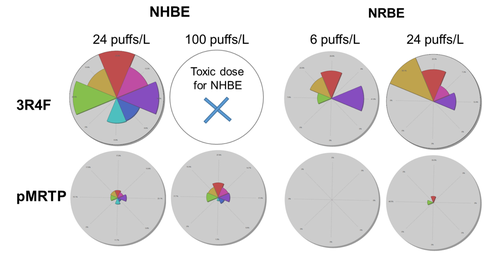
Volcano plots represent the global differentially expressed genes (light blue dots: down-regulated; yellow dots: up-regulated; dark blue dots: below fdr p-value of 0.05). Four hours exposure to the same dose of TPM from the 3R4F resulted in twice as many differential expressed genes in NHBE cells compared to NRBE exposed cells. The exposure of cells to the same dose of pMRTP (24 puff/L) resulted in a weak transcriptional response in both species.

Biological processes perturbed in healthy systems



Using a computational approach based on causal models of tissue-specific biological networks[5-8], gene fold-changes were translated into a quantitative measure for the amount of the impact that one network is perturbed when exposed to an external stimuli[9]. For human 46 and for rat 43 subnetworks (indicated by numbers on the networks) were calculated for their level of perturbation. Hu: human.

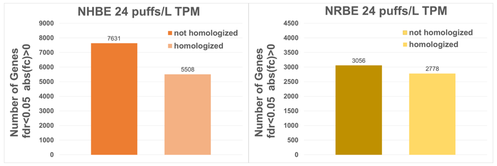
Impact on biological processes



The computational modeling approach identified proliferation, apoptosis, and senescence as the most perturbed molecular processes after 4 hours exposure of NHBE cells to 3R4F TPM. Cell stress network was impacted to a lesser extent. In NRBE cells the most perturbed biological process was cell stress, followed by proliferation and senescence. Lower perturbation amplitudes of these processes were observed after exposure to pMRTP in both species.

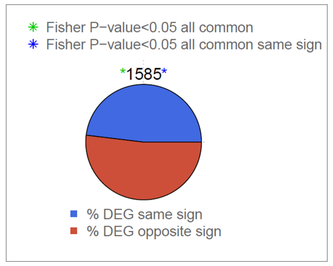
Translational approach of the effect of 3R4F TPM across species

Homologization between human and rat genes



For a cross-species translational approach genes need to exist in both species. Fro creating a common set of genes, the gene symbols were mapped between both species. Homologizing the genes between rat and human reduces the number of genes to be used for translational approach. 2100 human differential expressed genes (DEG, fdr<0.05 and abs(fc)>0) did not have a counterpart in the rat and 300 of the rat DEG genes do not have a counterpart in the human by gene symbol.

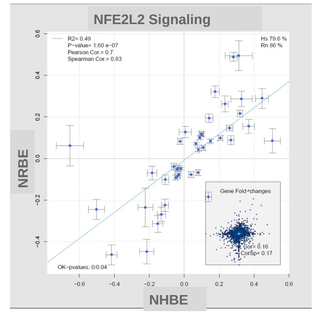
Common DEG between human and rat and their direction



From the 5508 DEG in human and the 2778 DEG in rat 1585 genes were in common to both species. However, more than the half were regulated with opposite direction following a 4 hour-exposure to 3R4F TPM.

networks perturbed in both species	R2	Spearman Correlation factor	Pearson Correlation factor
Cell Stress / Xenobiotic Metabolism Response	0.49	0.66	0.7
Cell Stress / NFE2L2 Signaling	0.49	0.83	0.7
Cell Proliferation / Cell Cycle	0.39	0.78	0.62
Cell Proliferation / Growth Factor	0.26	0.53	0.51
Inflammation / Mucus hypersecretion	0.09	0.19	0.3
Cell Stress / Osmotic Stress	0.03	0.09	0.18
Senesence / Regulation by tumor suppressors	0.03	0.74	0.17

Species translation on network level



Scatter plot shows the network values (main graph) and gene expression fold changes (inset) of the intersection of the human and rat «NFE2L2 signaling» sub-network perturbed after 3R4F TPM exposure (24 puffs/L). R2 corresponds to the coefficient of determination in relation to the linear regression fit (LM fit). Blue line shows the linear regression lines computed by least squares fit. Cor.: Correlation. For gene expression fold changes, Cor and CorSp correspond to Pearson and Spearman correlations, respectively. Horizontal and vertical error bars correspond to the 95%-confidence interval of each human and rat backbone node (blue dot) score, respectively.

At the network level, 41 subnetworks were in common between human and rats. Of these, 24 networks were significantly perturbed using the NHBE derived data set, but only 8 subnetworks were perturbed in rats. From these 8, 7 subnetworks (see table) were the same in both species. 4 subnetworks showing a correlation between human rat biology. The cell stress sub-network NFE2L-signaling showed the highest correlation with a factor R2=0.49. Network level comparison showed a higher correlation than on gene expression level.

Conclusion

The quantitative systems toxicology approach utilizing causal network models representing key biological mechanisms is a powerful tool to analyze the effect of conventional cigarette or of MRTPs exposure. Here, we provide mechanistic insight of 3R4F or pMRTP TPM-induced biological impact on key cellular processes in NHBE and NRBE cells. Overall, the results indicate a reduced biological network perturbation of pMRTP compared to 3R4F. A better understanding of the range of applicability of the translational concept is important to increase the predictability of signaling responses as well as increase the confidence in the estimation of human risk from rodent data in the context of toxicological risk assessment. In this study, the demonstrated translatability of the investigated biological processes was relatively low. NRBE cells responded to the same concentration of 3R4F TPM with much less differential expressed genes. Interestingly, NHBE respond to 3R4F TPM exposure with a high perturbation of the proliferation network whereas the NRBE exposure resulted in a high perturbation of cell stress. This may reflect the high amount of opposite directionality seen in the common homologized genes. Only few subnetworks were perturbed in exposed NRBE cells. In summary, after 3R4F TPM exposure the highest species correlation was found for the cell stress sub-network NFE2L2 signaling. The correlation between TPM-induced perturbation in human and rat bronchial epithelial cells is higher on network level than on DEG level, suggesting that approaches that aim to translate biology from one species to another should use pathway and network levels.

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PMI RESEARCH & DEVELOPMENT

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