Proteomics as part of a multi-omics systems toxicological assessment of a mentholated candidate modified risk tobacco product

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Introduction and Objectives

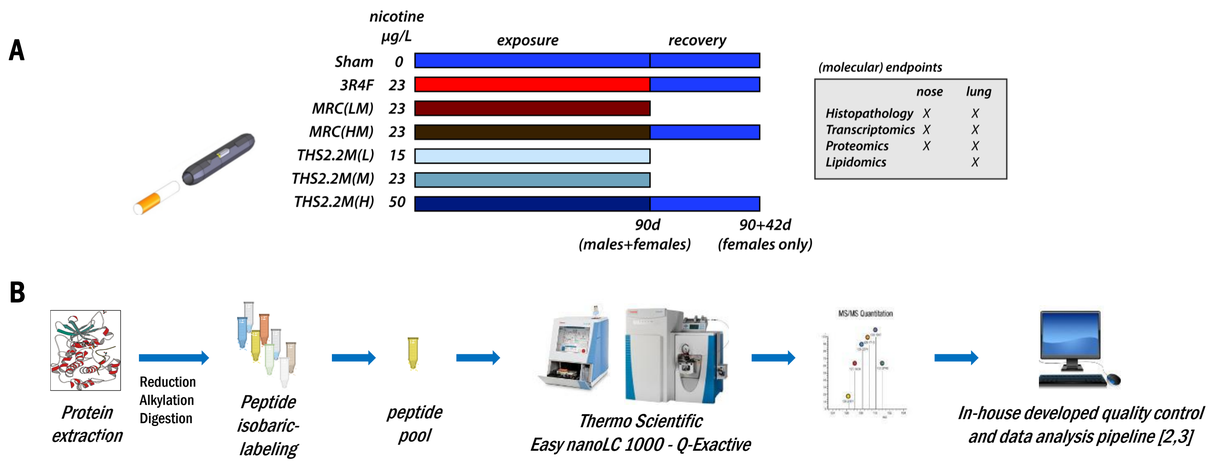
Modified risk tobacco products (MRTPs) are being developed with the aim of reducing smoking-related health risks. The Tobacco Heating System 2.2 (THS2.2) is a candidate MRTP that uses the heat-not-burn principle. We engaged a multi-omics systems toxicology approach to assess the respiratory effects of a mentholated THS2.2 (THS2.2M) in a 90-day rat inhalation study (OECD test guideline 413). For this, we complemented the standard OECD-endpoints by quantitative proteomics (iTRAQ) and transcriptomics of nasal epithelium and lung tissue. Quantitative causal network, functional association network, and gene set analyses of the transcriptomics and proteomics data facilitated the identification and comparative assessment of the exposure effects.

The adaptive response of the nasal epithelium to cigarette smoke (CS) included squamous cell metaplasia and an inflammatory response, with high correspondence between the molecular and histopathological results. In contrast to CS exposure, the adaptive tissue and molecular changes to THS2.2M aerosol exposure were much weaker and were limited mostly to the highest THS2.2M concentration in female rats.

In the lung, CS exposure induced an inflammatory response, triggered cellular stress responses, and affected sphingolipid metabolism. These responses were not observed or were much lower after THS2.2M aerosol exposure.

Overall, this multi-omics system toxicology analysis – including quantitative proteomics – complemented and reconfirmed the results from the classical toxicological endpoints as well as identified reductions in a range of additional toxicological pathways [1].

Methods



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Analysis	samples	each iTRAQ set	#sets
Set1	all 90d samples	7 sample types + Refmix	12
Set2	90+42d and corresponding 90d samples	8 sample types	6

Sample and channel assignments randomized

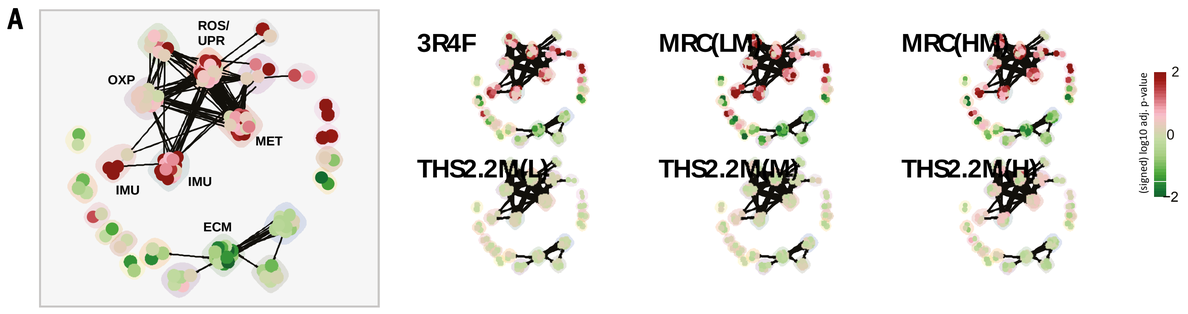
Figure 1. ***Design of the 90-day systems toxicology study to assess effects of CS and THS2.2M exposures on rat respiratory organs.***

A. Groups of male and female rats were exposed for 90 days to fresh air (Sham) or cigarette smoke of three reference cigarettes: a standard reference cigarette (3R4F), and a low menthol (MRC(LM)) and high menthol (MRC(HM)) reference cigarette, all at 23 µg nicotine/L. Groups of rats were exposed to aerosol from THS2.2M (15, 23, and 50 µg/L nicotine). The 90-day exposure period was followed by a 42-day recovery period with female rats exposed to fresh air. Rats were exposed for 6 h per day, for 5 days per week. N=6 for each group.

B. Quantitative proteomics workflow [2,3].

C. Definition of iTRAQ analysis sets.

Results – Lung tissue



ROS = oxidative-stress response; UPR = unfolded-protein response; OXP = oxidative phosphorylation; MET = metabolism; IMU = immune-related; ECM = extracellular matrix

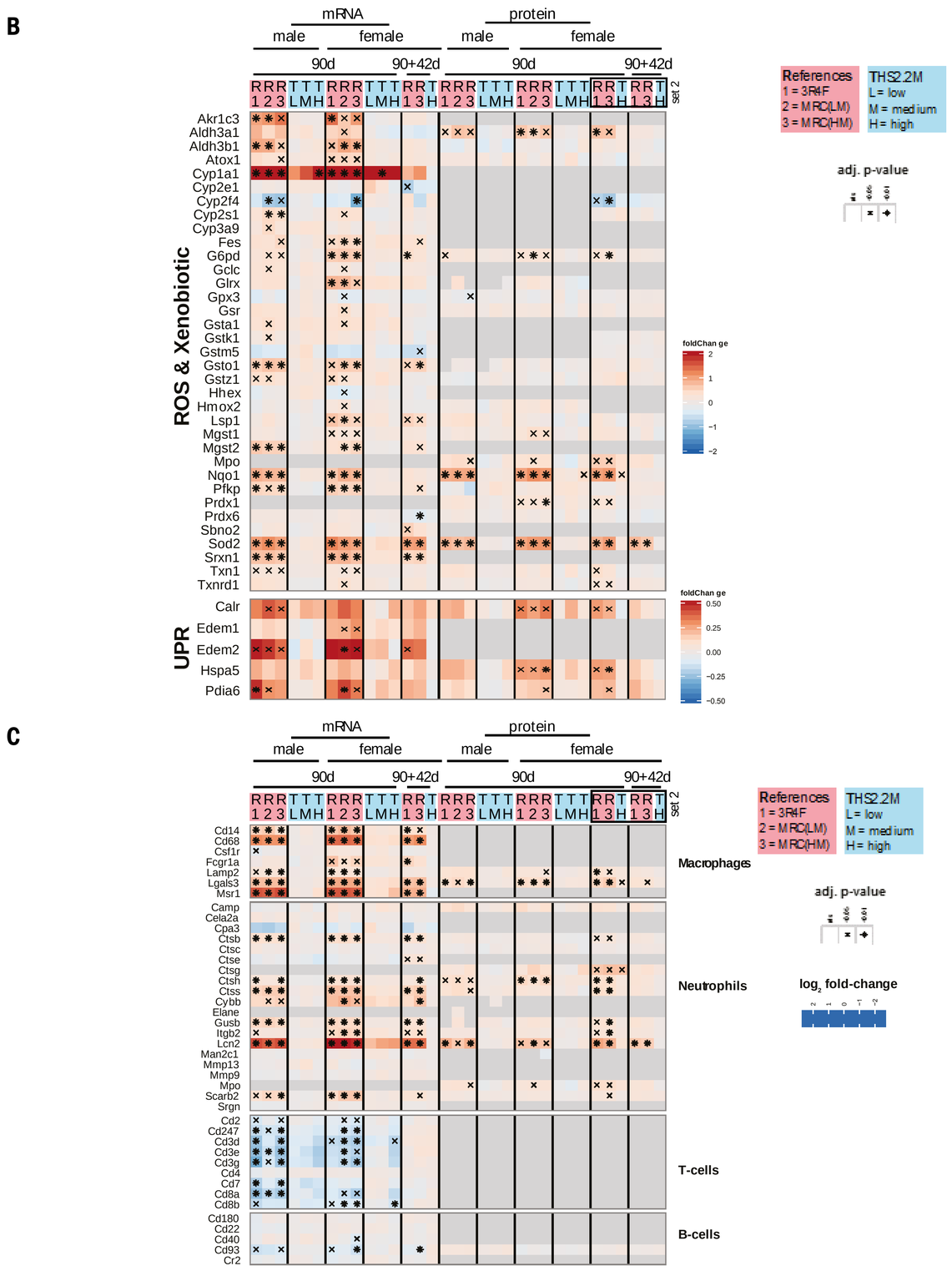


Figure 2. ***Response of the lung to exposure.***

A. Functional association clustering for proteomics data of the lung tissue. Functional clusters affected by the exposure conditions were identified and compared across the different clusters, here the 90d female data are shown.

B. Expression/abundance profiles for cellular stress-related gene/protein sets.

C. Expression profiles for panel of immune-cell markers.

Results – Nasal Epithelium

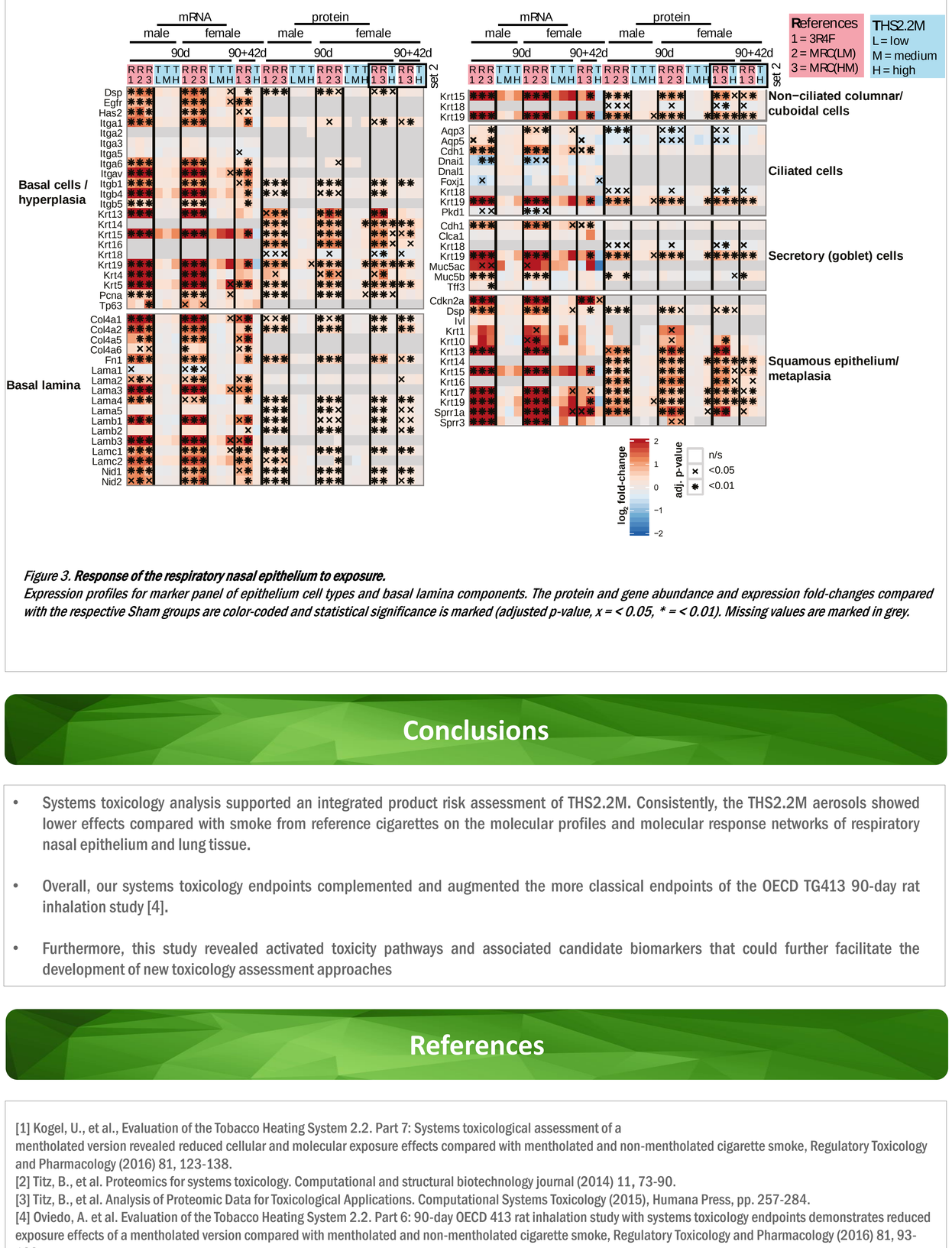


Figure 3. ***Response of the respiratory nasal epithelium to exposure.***

*Expression profiles for marker panel of epithelium cell types and basal lamina components. The protein and gene abundance and expression fold-changes compared with the respective Sham groups are color-coded and statistical significance is marked (adjusted p-value, x = < 0.05, * = < 0.01). Missing values are marked in grey.*

Conclusions

- Systems toxicology analysis supported an integrated product risk assessment of THS2.2M. Consistently, the THS2.2M aerosols showed lower effects compared with smoke from reference cigarettes on the molecular profiles and molecular response networks of respiratory nasal epithelium and lung tissue.
- Overall, our systems toxicology endpoints complemented and augmented the more classical endpoints of the OECD TG413 90-day rat inhalation study [4].
- Furthermore, this study revealed activated toxicity pathways and associated candidate biomarkers that could further facilitate the development of new toxicology assessment approaches

References

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Competing Financial Interest

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