Cigarette smoking is a strong causative element contributing to the development of several diseases, including cardiovascular (CVD) and chronic obstructive lung diseases such as COPD/emphysema. Reliable system-wide measurements are key to a meaningful systems-toxicology approach by which impact of toxicants in cells are evaluated (Hoeng et al., 2012). We evaluated the advantages of using proteomics methodologies for the product assessment of candidate modified risk tobacco product (cMRTP), which is already commercialized, and prototype modified risk tobacco product (pMRTP), which is still under further development. The objective was to integrate the proteomics data with other endpoints, mainly transcriptomics and lipidomics datasets, within our systems toxicology approach. Two studies were conducted on 2 different mice strains: 1) an ApoE-deficient mice which are prone to developing premature atherosclerosis and emphysema (using pMRTP). We conducted on 2 different mice strains: 1) an ApoE-deficient mice which are prone to developing premature atherosclerosis and emphysema (using pMRTP). We evaluated the effects of combusting cigarette smoke (CS) from a reference conventional cigarette (3RF) and aerosol from two different types cMRTP and pMRTP on mice exposed for up to 7/8 months.

RESULTS

Figure 2. Volcano plots representing the proteome response profiles in lungs for the ApoE-/- study. For each protein, the protein expression change, calculated as the log2 fold change, is plotted on the x-axis and the statistical significance, proportional to the negative log10-adjusted p-value, is plotted on the y-axis. Yellow and blue dots highlight proteins that are significantly up- or down-regulated, respectively, compared with the sham group at each respective time point (Bergeron-Hofschlag adjusted p-value <0.05).

Figure 3. Functional clusters impacted by exposure to conventional cigarette smoke in the ApoE-/- study. Functional protein network for differentially expressed proteins upon 3 months of 3RF exposure was identified with the dnet approach to highlight regulated biological functions (Fang et al.). Each node in the network corresponds to a regulated protein, each edge to a functional link reported in the String database (Manavsvan et al., 2013). Clusters of regulated protein functions were identified and functionally annotated. Node colors show the significance of regulation upon 8 months of cigarette smoke (3RF) exposure (maximum signed log2 adjusted p-value <0.05).

Figure 4. Volcano plots representing the proteome response profiles in lungs for the C57BL/6 study. For each protein, the protein expression change, calculated as the log2 fold change, is plotted on the x-axis and the statistical significance, proportional to the negative log10-adjusted p-value, is plotted on the y-axis. Yellow and blue dots highlight proteins that are statistically significantly up- or down-regulated, respectively, compared with the sham group at each respective time point (Bergeron-Hofschlag adjusted p-value<0.05).

Figure 5. Functional clusters impacted by exposure to conventional cigarette smoke for the C57BL/6 study. Functional protein network of consistently up- (A) or down- (B) regulated proteins upon 7 months of 3RF exposure highlighted regulated biological functions. Each node in the network corresponds to a regulated protein, each edge to a functional link reported in the String database (Manavsvan et al., 2013). Clusters of regulated protein functions were identified and functionally annotated. Node colors show the significance of regulation upon 7 months of cigarette smoke (3RF) exposure (maximum signed log2 adjusted p-value <0.05).

Figure 6. Comparison of exposure response in ApoE-/- and C57BL/6 studies. (A) Protein expression ratio for most strongly regulated proteins in ApoE-/- study with comparison to response in C57BL/6 study. Differentially expressed proteins (|log2 fold-change| >1.5 and adj.p<0.05) are depicted as red and green dots for up-regulated and down-regulated proteins, respectively. (B) Functional protein cluster representing the response to the last (4th) time point (120 days) for the ApoE-/- and C57BL/6 study.

Figure 7. Response of proteins implicated in reactive oxygen species responses. The volcano plot of all significantly regulated proteins is shown. The x-axis represents the log2 fold-change and the y-axis represents the false discovery rate (FDR)-adjusted p-value. The gene ontology terms for ‘Response to oxidative stress’ and ‘Response to hydrogen peroxide’ are highlighted. Significant changes are highlighted in red.

Figure 8. Surfacing proteins and surfactant lipid responses in C57BL/6 and ApoE-/- mice. CS exposure significantly affected both protein (A) and lipid (B) components of surfactant, while MRTP exposure did not induce similar changes and the cessation and switching groups rapidly returned to sham levels of these proteins and lipids.

CONCLUSIONS

- Quantitative proteomics of lung tissue captured the important biological process that are associated with smoking-induced diseases such as nicotine resistance, oxidative stress, immune response and metabolic alterations.
- Generated proteomics datasets showed reproducibility and robustness between the 2 mice strains in the 2 different studies.
- Exposure to 3RF smoke induced a strong effect in a time-dependent manner with significant changes in differential protein expressions in both the ApoE-/- and C57BL/6 mice, whereas switching to and continuous exposure to both cMRTP did not result in decrease or no change in the differential protein expressions of, reaching similar levels of cessation in both the ApoE-/- and C57BL/6 mice.
- Integration of datasets with other data modalities such as lipidomics showed its success in verifying the surfactant-related protein and lipid response (Figure 8).

- The integration of proteomics datasets with transcriptomics, lipidomics and histopathological datasets proved to be valuable in the systems toxicology approach to perform product assessment of MRTPs and reduced risk products (RRPs).

REFERENCES