A Mechanistic Study using 2D-PAGE Proteomic Approach to investigate the Effect of Cigarette Smoke-Induced COPD, Cessation and Switching to modified risk tobacco product in the lungs of C57BL/6 Mice

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P-561.00

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is defined by the World Health Organisation (WHO) as a lung disease characterized by chronic obstruction of lung airflow that interferes with normal breathing and is associated with narrowing of the small airways, chronic bronchitis, and the development of alveolar emphysema. Cigarette smoke is the primary risk factor in the development and progression of COPD. PMI is developing potentially modified risk tobacco products (MRTPs) in effort to reduce the risk of smoking-related disease in smokers who switch from combustible cigarettes to the MRTP. In this study, the impact on the development of emphysema/COPD following inhalation of aerosol from two tobacco products, a reference cigarette (3R4F, University of Kentucky) and a prototypic modified risk tobacco product (pMRTP), was evaluated in C57Bl/6 mice over a period of 7 months. After 2 months of exposure to 3R4F, switching and cessation groups were exposed to pMRTP aerosol or filtered air (sham), respectively. 2D-PAGE method was used for relative quantification of differentially expressed proteins in lung of C57BL/6 mice.

The aim of the study is to use the 2D-PAGE:

- To determine how suitable is 2D-PAGE proteomics approach for product assessment in *in vivo*;
- To identify differentially expressed proteins in the different experimental groups;
- To determine the effects of pMRTP and switching to pMRTP in comparison to Sham.

MATERIALS & METHODS

The mice were exposed to an aerosol from 3R4F (750 micrograms/liter of total particulate matter – TPM), pMRTP or filtered air for 4 hours per day, 5 days per week, up to 7 months. Aerosols from both tobacco products used had the same nicotine concentration - 34.4 micrograms/liter. After 2 months of exposure to 3R4F, switching and cessation groups were exposed to pMRTP aerosol or filtered air, respectively. Right lung samples (118 total) from month 1, 3, 5 and 7 were analyzed.

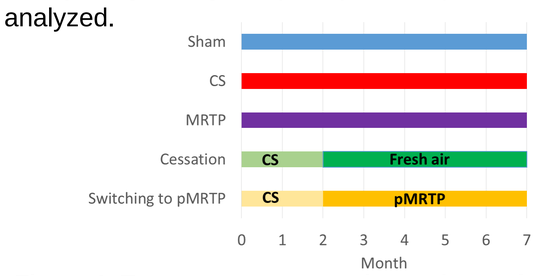

Figure 1. Treatment groups and experimental design

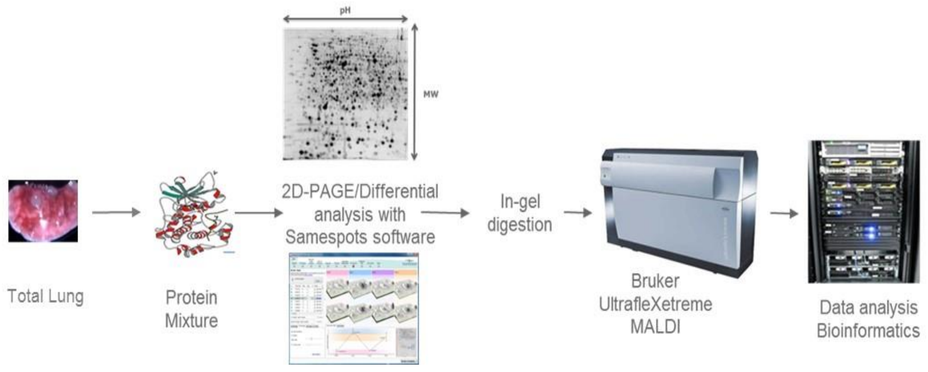

Figure 2. 2D-PAGE workflow used for the identification of differentially expressed proteins.

Procedure:

2D-PAGE: 150 µg of protein was loaded and separated on 11 cm strip, 3-10 NL, then on13 cm 12% SDS-PAGE and finally stained with Sypro Ruby.

Differential analysis: SameSpots software (TotalLab) was used for the detection of differentialy expressed proteins by comparison to the control sample (Sham).

Protein identification: Tryptic digested samples were analyzed using MALDI TOF/TOF-MS and were identified using Mascot search engine against the Uniprot Mouse database.

RESULTS

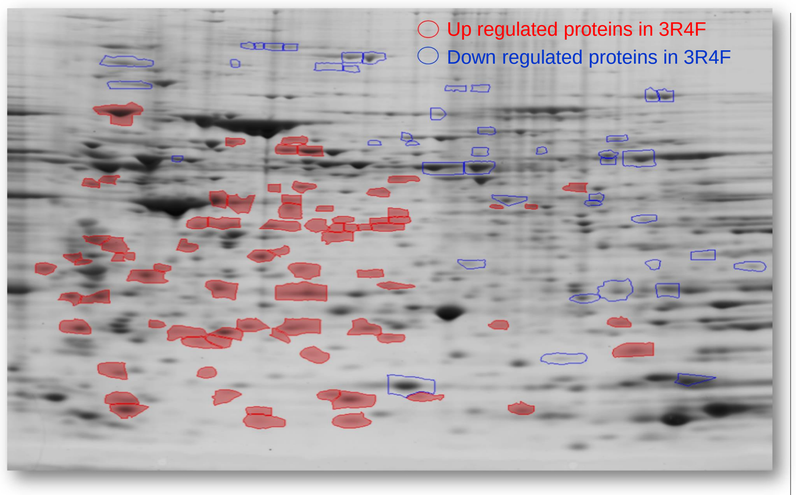

Figure 3: Representative 2D-PAGE (IEF: pH 3-10 NL, 11 cm/ 12% Bis-Tris 13 cm precast gel) image of the 3R4F sample Month 7 with the differentially expressed proteins detected in comparison with Sham.

Figure 4: Volcano plot of the 2D-PAGE data for the comparisons of all the treatment group with control group. Blue and yellow dots are respectively down- and up-regulated proteins with a raw p-value of ≤ 0.05 (Anova).

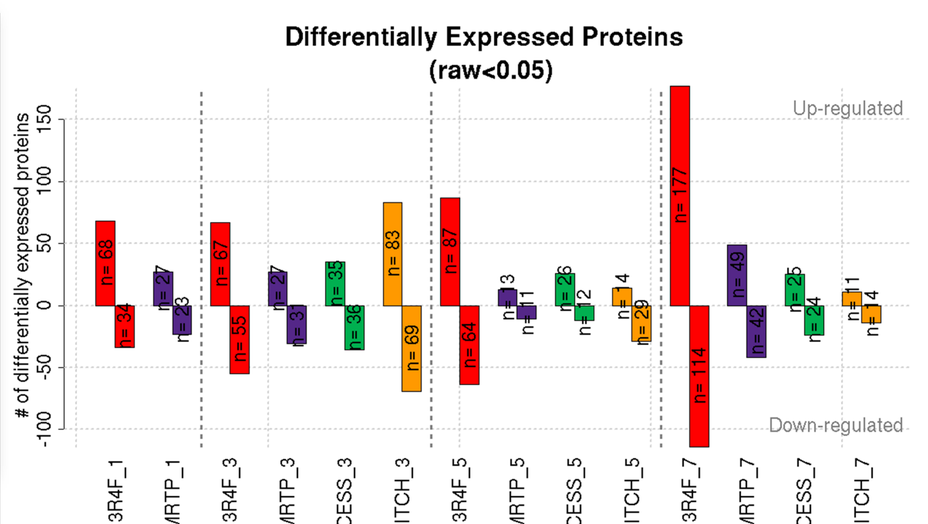

Figure 5: Distribution of the differentially expressed proteins according to individual group comparison to Sham with raw p-value ≤ 0.05 (Anova) and with a fold change ≥ 1.2 throughout time point

Protein Accession number	Protein name	Gene name	Anova (p-value)	Fold
P18242	Cathepsin D	Ctsd	6.3E-08	5.0
P24452	Macrophage-capping protein	Capg	1.3E-03	2.0
D3Z220	Dipeptidyl-peptidase 1	Ctsc	1.2E-03	2.2
P35242	Pulmonary surfactant-associated protein A	Sftpa1	0.003	2.4
P63028	Translationally-controlled tumor protein	Tpt1	0.0003	- 2.6
Q62151	Advanced glycosylation end product-specific receptor	Ager	0.0089	- 2.0
Q64727	Vinculin	VCl	8.9E-05	- 1.9
Q9EQ20	Methyl malonate semialdehyde dehydrogenase (acylating), mitocondrial	Aldh6A	1.4E-03	- 1.5
F8VQ05	Protein Fryl	Fryl	7.5E-04	- 1.7
P08228	Superoxide dismutase (Cu-Zn)	Sod 1	1.3E-03	- 1.5

Table 1: List of identified differentially regulated proteins from the comparison 3R4F to Sham; using 2D-PAGE. Protein were identified using MALDI TOF-TOF-MS.

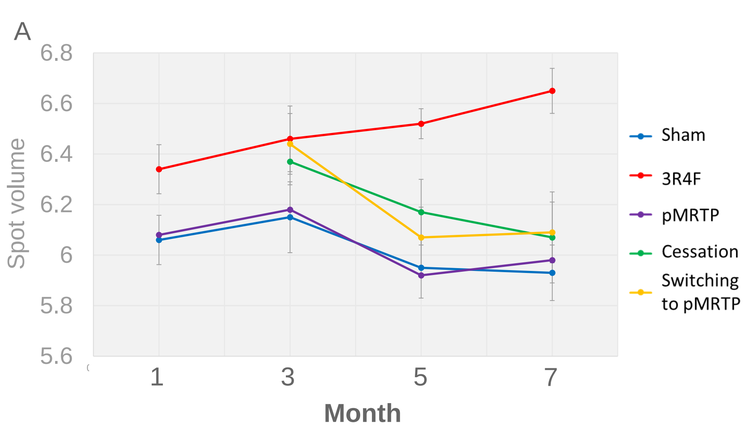

Figure 5: A: Expression profile of Cathepsin D among groups and time points using 2D-gel analysis.
B: 3D representation of Cathepsin D spot.
C: Expression profile of Cathepsin D among groups and time points using iTRAQ analysis.

Conclusions

- The implementation of 2D-PAGE/MALDI TOF/TOF-MS resulted in the identification of **130 proteins** differentially regulated proteins in the different treatment groups and time points illustrated the suitability of the technique for product assessment.
- The 2D-PAGE/MALDI TOF-TOF datasets were used to complement the iTRAQ LC MS/MS datasets on the proteins that were differentially expressed **(Figure 5)**. Both datasets were further verified using reverse phase protein array (RPPA) and transcriptomics datasets (data not shown) on selected protein targets that showed the largest fold change.
- Datasets are showing that exposing mice to pMRTP have illustrated less differentially expressed proteins as compared to 3R4F for all the time points **(Figure 4).**
- Switching to pMRTP showed that at month 7, the number of differentially expressed proteins is close to the cessation groups **(Figure 4).**

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HUPO Conference, Madrid, Spain
October , 2014.