Using 2D-PAGE and iTRAQ Proteomics Approaches to Investigate the Effect of Cigarette Smoke Fraction Exposure on Primary Normal Human Bronchial Epithelial (NHBE) Cells

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Introduction

Cigarette smoke (CS) is a complex mixture of chemicals eliciting oxidative stress and inflammation, which induces smoking-related disorders in respiratory tissues and cells. In order to advance our knowledge regarding the impact of cigarette smoke on the human bronchial system, a system biology approach was developed within Philip Morris International (PMI) that combines proteomics, together with gene expression analyses. Primary normal human bronchial epithelial (NHBE) cells were exposed for 24 h to a solution of phosphate-buffered saline through which the smoke from reference cigarette (3R4F) had been bubbled (i.e. smoke-bubbled PBS or sb PBS). The dose applied was selected based on survival assay results showing less than 20% cytotoxicity after 24 h exposure.

The aim of the study was to use the 2D-PAGE and iTRAQ approaches:

(i) To identify regulated proteins from both approaches.

(ii) To determine which biological or molecular function processes are impacted.

(iii) To compare data with gene expression datasets generated within PMI.

(iv) To determine the feasibility of using the 2D-PAGE and iTRAQ approaches for product assessment of new modified risk tobacco products (MRTPs) using NHBE cells as a model cell system.

MATERIALS & METHODS

The seeding density 75,000 cells/well of the NHBE cells was used a 12-well-plates. NHBE cells were incubated for 24 h in sbPBS 3R4F solution (0.033 puffs/mL or 3.1 cigarette/L), followed by protein extraction as described in **Figure 1.** The proteomics approaches used in this study are summarized in **Figure 2.**

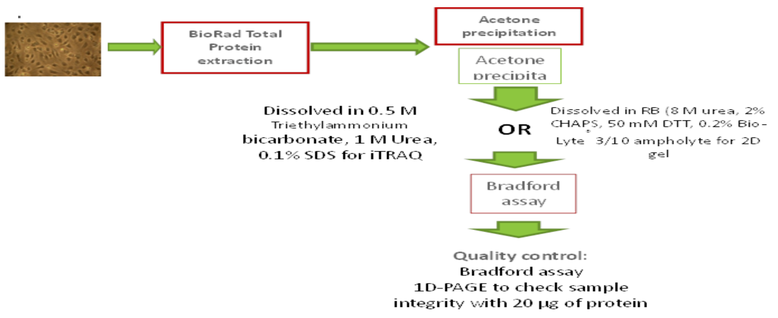


Figure 1: Workflow for the extraction of proteins from NHBE cells to be prepared for 2D-PAGE and iTRAQ analysis.

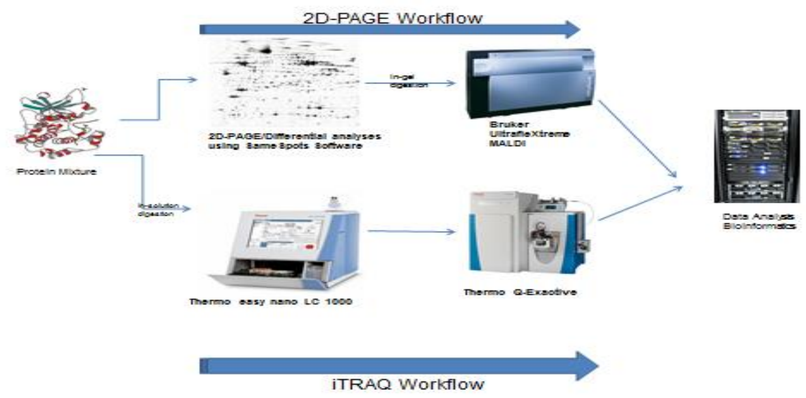


Figure 2: 2D-PAGE and iTRAQ workflows showing the instruments used for the identification of differentially expressed proteins. In NHBE exposed cells.

Bioinformatics.

2D-PAGE: SameSpots software™ (TotalLab) was used for the detection of differential proteins by comparison to the control sample. Proteins were identified using Mascot search engine against the uniprot human database.

iTRAQ: Protein were quantitated using Proteome Discoverer™ software (ThermoScientific) against a reference sample. Proteins were identified using a combination of Mascot and Sequest search engines against the uniprot human database.

RESULTS

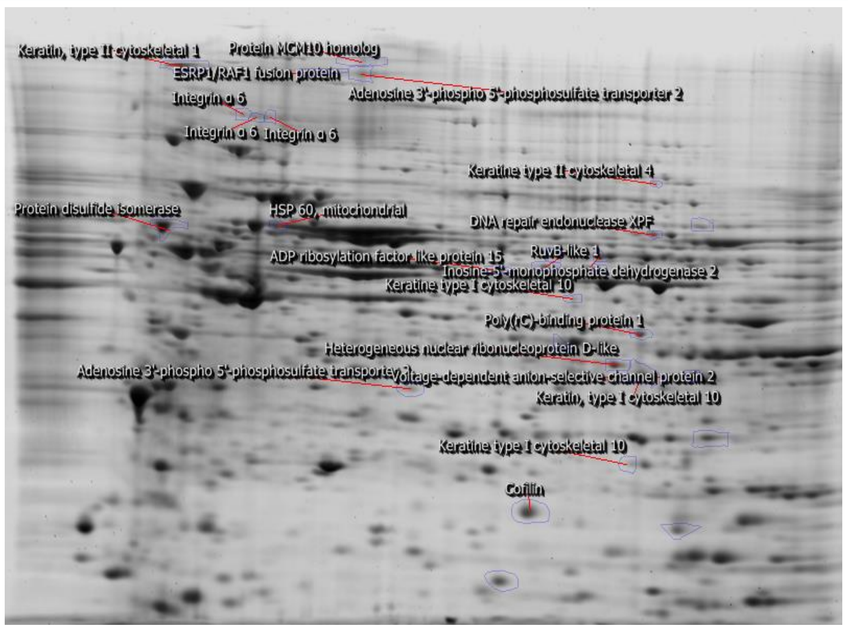


Figure 3: Representative 2D-PAGE image of the 3R4F sample with the identified differentially expressed proteins(150 µg of protein loaded, 11 cm strip, 3-10 NL and stained with Sypro Ruby). Proteins were identified using Bruker MALDI TOF/TOF.

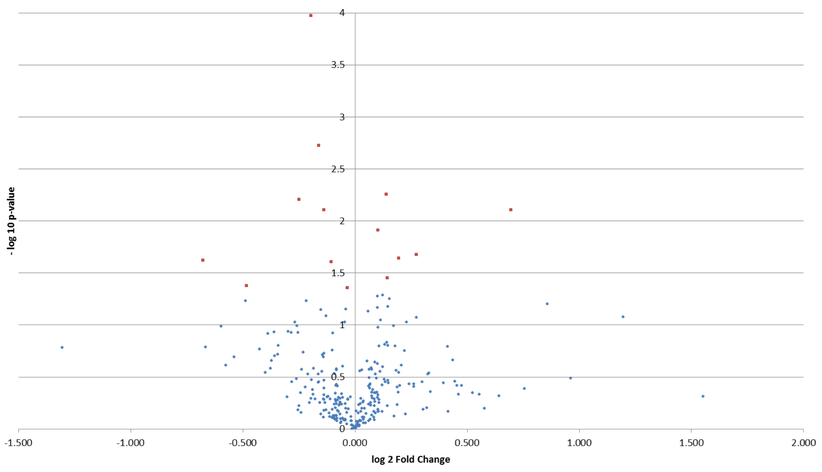


Figure 4: Volcano plot of the 2D-PAGE data for the comparison of 3R4F to control. Red dots are proteins that had p-value of <0.05.

A

Accession number	Protein name	3R4F/Sham	Peptide count
Category I			
P09601	Heme oxygenase 1	4.12	1;2;1
P04264	Keratin, type II cytoskeletal 1	1.84	6;1;2
Q9Y3L3	SH3 domain-binding protein 1	1.69	1;1;1
Q04828	Aldo-keto reductase family 1 member C1	1.69	1;1;1
P46779	60S ribosomal protein L28	1.51	1;2;2
Category II			
Q13501-2	Isoform 2 of Sequestosome-1	1.97	1;-;1
P35527	Keratin, type I cytoskeletal 9	1.67	4;-;2
P35613-2	Isoform 2 of Basigin	1.53	1;1;-
Category III			
B4DL24	DNA-directed RNA polymerase III subunit RPC5	1.85	-;3;-
Q9BVK6	Transmembrane emp24 domain-containing protein 9	1.56	-;1;-
H0YNE3	Proteasome activator complex subunit 1	1.55	-;1;-

B

Accession number	Protein name	3R4F/Sham	Peptide count
Category I			
Category II			
P68431	Histone H3.1	0.63	2;-;1
P13645	Keratin, type I cytoskeletal 10	0.63	-;2;3
Category III			
C9J0K6	Sorcin	0.66	1;-;-
O14980	Exportin-1	0.65	1;-;-
P35637-2	Isoform Short of RNA-binding protein FUS	0.63	1;-;-
P07996	Thrombospondin-1	0.54	-;1;-

Table 1: Tables of identified regulated proteins from comparison of 3R4F to control (A: up-regulated, B: down-regulated) using iTRAQ LC MS/MS workflow. Protein were identified using Q-Exactive.

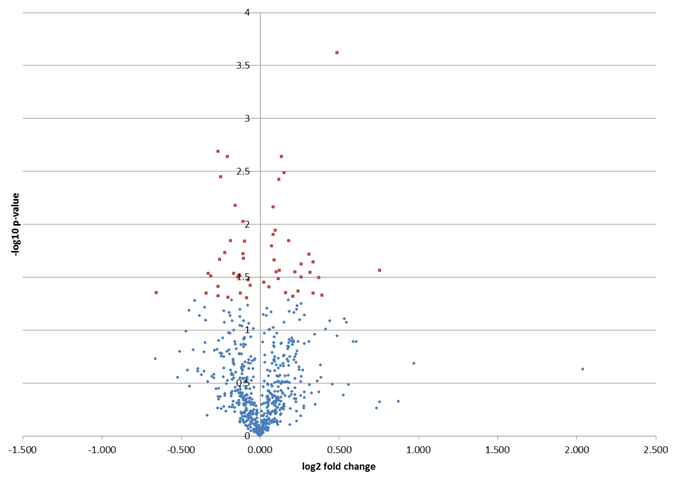
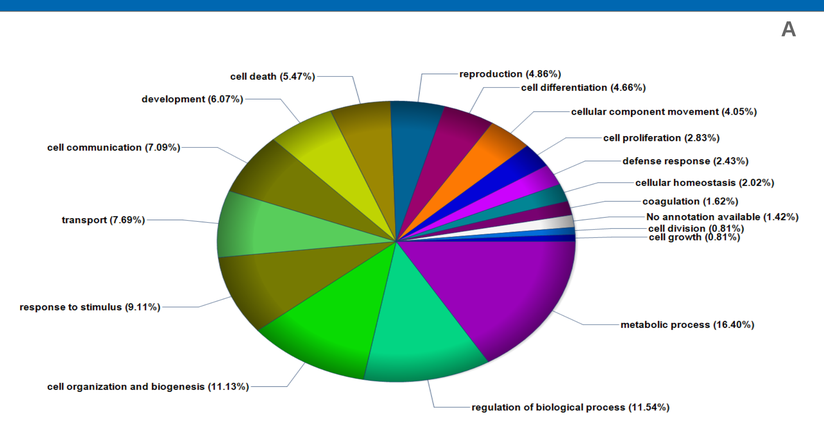


Figure 5: Volcano plot of the iTRAQ data for the comparison of 3R4F to control. Red dots denote proteins with a p-value <0.05.

A



B

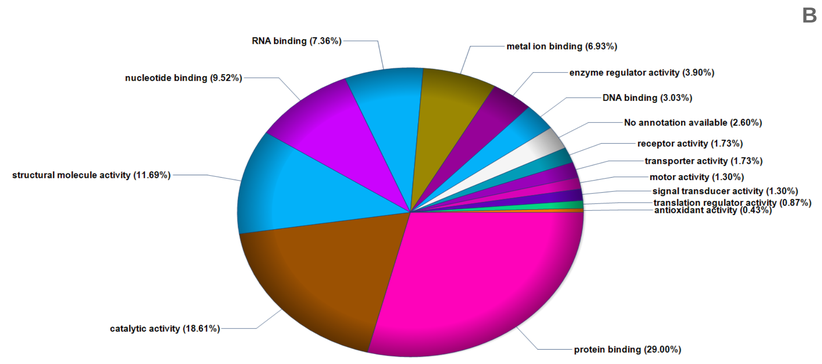


Figure 6: A) Biological processes, B) Molecular function impacted from regulated proteins as a result of exposure to 3R4F (iTRAQ datasets).

Accession	Protein Name	Gene Name	Fold (Transcriptomics)	Fold (Proteomics)
P09601	Heme Oxygenase 1	HMOX1	3.53	4.12 (iTRAQ)
Q04828	Aldo-keto reductase	AKR1C1	1.60	1.69 (iTRAQ)
P31947	14-3-3	SFN	0.10	1.48 (iTRAQ)
P23229	Integrin α 6	ITGA6	-0.45	-0.67 (2D-PAGE)
Q13501	Sequestosome-1	SQSTM1	1.97	1.97 (iTRAQ)

Table 2: Representative candidate proteins that were matched to the generated transcriptomics datasets based on matching to gene name.

Conclusions

- The implementations of 2D-PAGE and iTRAQ workflows for identification of differentially expressed proteins as a result of exposure to 3R4F in NHBE cells was successful.
- With iTRAQ workflow, more proteins were identified compared to the 2D-PAGE approach. But the 2D-PAGE approach can complement and verify proteins identified by iTRAQ.
- The majority of the identified regulated proteins impacted the protein binding and catalytic activity of the NHBE cells as a result of exposure to 3R4F smoke.
- Multiple differentially expressed proteins from 2D-PAGE and iTRAQ datasets were matched to in-house transcriptomics datasets, showing the same trend in regards to the regulation.
- 2D-PAGE and iTRAQ approaches may be used for future assessment of MRTPs developed within PMI to demonstrate harm reduction using NHBE cell model..

REFERENCES

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