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Evaluation of biological reproducibility in differential proteomics analysis of primary and secondary nasal polyposis by iTRAQ™

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High throughput differential proteomics analysis on human samples is an important approach to unmask key proteins in the pathogenesis of a disease. However, these analyses are known to often highlight biological differences between individuals rather than differences related to the disease.

The aims of this study were to evaluate the proportion of proteins which are independent of genetic background over ten patients affected by nasal polyposis (NP) and to explore the role of these proteins in NP pathogenesis by network analysis.

We chose to compare NP related to cystic fibrosis (CF) genetic disease (CF-NP, also called secondary NP) to primary NP of unknown origin (nonCF-NP). We used primary cultures of human nasal epithelial cells from five nonCF-NP and five CF-NP patients.

In each experiment total proteins from one nonCF-NP patient and one CF-NP patient were randomly paired and total proteins were labelled with isobaric tagging reagents (iTRAQ™), analysed by NanoHPLC-MALDI-TOF-TOF MS and quantified by Mascot v2.2. The relevant differentially expressed proteins were analysed using Ingenuity.

We identified on average 727(±177) proteins in the five independent experiments with confidence and quantified 53(±5)% of them. In each experiment 5(±2)% of the quantified proteins was upregulated, 28(±6)% down-regulated and 67(±7)% did not change. The differential regulation of some of these proteins was tested by immunoblots.

We analysed closely the set of proteins quantified with high reproducibility to evaluate the level of biological relevance of this study. A total of 163/267 showed exactly the same quantification trend across patients. This suggests that 61% of the quantified proteins could be directly linked to the differences between CF-NP and nonCF-NP (bio-relevant set), and 39% to inter-individual variability (background set).

A network analysis shows the high degree of interaction of the bio-relevant protein set with CFTR, the mutated protein responsible for CF. On the contrary, the background set has a very low degree of connection with CFTR, suggesting that fluctuating variation of these proteins are more related to genetic background than to the cause of NP.