Institutionen för Medicin, Enheten för Lungmedicin

Quantitative Proteomic Approaches for the Analysis of Human Lung Samples in Pulmonary Sarcoidosis

AKADEMISK AVHANDLING

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ABSTRACT

This thesis is focused on the analysis of protein expression profiles from the airways of sarcoidosis patients using quantitative proteomics. Sarcoidosis is a multisystemic inflammatory disorder of unknown etiology characterized by the presence of noncaseating granulomas in the affected organs. Previous findings in proteomics have reported identification of several proteins that certainly reflect the ongoing inflammation response but that did not have any specificity for sarcoidosis disease. The overall aim of these studies was therefore to continue searching for new protein candidates that could help us to determine the possible mechanisms behind sarcoidosis.

We first investigated the total protein profile in the lung lumen from two granulomatous disorders, sarcoidosis (HLA-DRB1*15 positive), characterized to present an unresolved chronic inflammation disease and chronic beryllium disease (CBD) by collecting the bronchoalveolar soluble proteins and applying differential gel electrophoresis (DIGE) coupled to mass spectrometry (MS) *(paper I)*. This led to the identification of fourteen proteins with altered profiles, some of these related to inflammation (β2-microglobulin, annexin II, complement C3, apolipoprotein A1, IgG kappa chain and heat shock protein 70) and the oxidative response (peroxiredoxin 5, hemopexin, α1-antitrypsin and superoxide dismutase), hence reflecting the persistent inflammation state in those granulomatous diseases.

Gel-based 2DE techniques have been claimed to be unreliable in quantitative proteomics. The introduction of differential gel electrophoresis **(**DIGE) in 2DE approches has greatly improves gel-to-gel variation and the reproducibility; however, other sources of variance have been highlighted. In a 2D experimental-related study, we investigated the different sources of variance that were intrinsic to gel-based proteomics. We measured the technical variance related to background subtraction algorithms [4-8%] and the experimental variance related to the 1st and 2nd dimension of 2DE workflow (~30%). In addition, we reported the improvement of the 4th generation image software program SameSpots^{TM} in terms of reduced levels of variance introduced from background algorithms, higher levels of accurate spot-matching and most importantly an improved objectivity of the analysis *(paper II)*.

To further evaluate the protein changes in sarcoidosis we investigated the protein profiles from purified alveolar macrophages (AM). With the intention to improve both the protein resolution we applied two complementary proteomic approaches. First, all soluble AM proteins were resolved using the DIGE technique *(paper III)*. Second all membrane-associated proteins (MAP) were then identified and quantified using the shotgun proteomic, liquid chromatography couple to mass spectrometry LC-MS/MS approach *(paper IV)*. We found similar results from these parallel studies, including several pathways altered in sarcoidosis (*papers III & IV).* In addition, by applying multivariate regression analysis we could also identify a robust model with a set of 13 proteins able to discriminate sarcoidosis from the healthy group *(paper IV)*.

Taken together, improvements in gel-based image software and clinical sample pre-treatment allow more accurate and quantitative analysis, revealing deeper insight into the proteome. The biological findings presented in this thesis give new perspectives in understanding AM and their role in sarcoidosis disease as well as the possibility to search for disease biomarkers.

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