Institute of Cell and Molecular Pathology

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Proteomic non-small cell lung carcinoma biomarker screening in bronchoalveolar lavage fluid

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Introduction

Non-small cell lung carcinomas (NSCLC) account for more than 80 % of all cases of lung cancer, the most common cancer worldwide in terms of both incidence and mortality. Diagnostic or prognostic molecular biomarkers may enable an earlier NSCLC detection as well as improved therapeutic monitoring.

Pilot study aim

Bronchoalveolar lavage fluid (BALF) taken during a mildly invasive routine bronchoscopic procedure provides a lung compartment-specific biofluid for NSCLC marker screening. The development of a method to reproducibly generate BALF proteome profiles to screen for NSCLC-related biomarkers might enable new diagnostic options regarding tumor detection and risk stratification.

Methods

For this pilot study, BALF was obtained from 6 NSCLC patients and 16 control cases. (Table 1)

	Number of patient samples	Mean age (years)
Non-small cell lung carcinoma (NSCLC)	6 (3 x squamous cell carcinoma, 3 x adenocarcinoma)	61 ± 16
Controls	16	57 ± 12

A MALDI-TOF mass spectrometry-based method was used to generate BALF proteome profiles. Cell-free BALF was desalted and fractionated using weak cation exchange functionalized superparamagnetic microparticles (Fig. 1). Proteome profiles were analyzed statistically for peak masses corresponding to NSCLC.

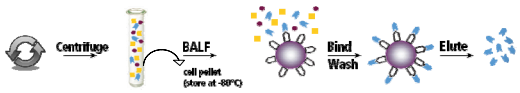
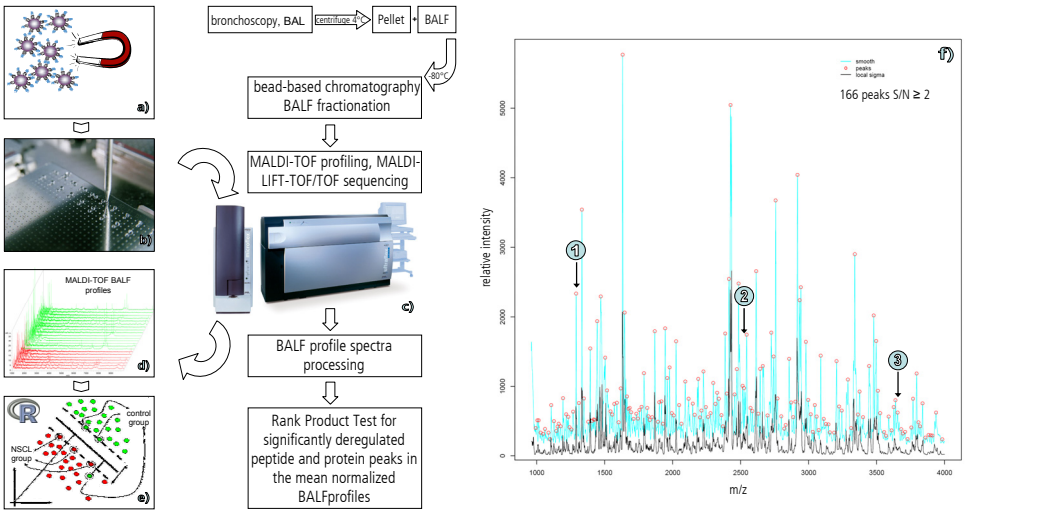


Fig. 1: Standardized sample preparation for MALDI-TOF analysis using magnetic bead technology by Bruker Daltonics.

Results

Comparative bioinformatic analysis of mass profiles showed BALF protein expression alterations in NSCLC patients. In the mass range 1 - 100 kDa, 20 peaks were found to be significantly upregulated in NSCLC patients compared to control cases. Peptide and protein identification revealed the significant upregulation of histatin 3 and calgranulin C, a sensitive marker of pulmonary inflammation.



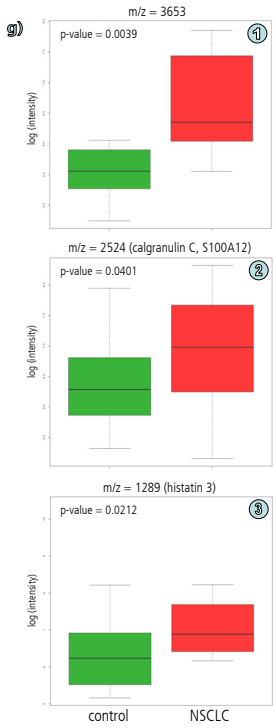


Fig. 2: Sample preparation using chromatographically functionalized magnetic beads [a)]; application of the eluted BALF peptides and proteins to an AnchorChip™ prestructured MALDI target plate (Bruker Daltonics) [b)]; acquisition of profile spectra up to 10 kDa using a highly sensitive linear MALDI-TOF device (Bruker MicroflexLT) and reflectron MALDI-TOF/TOF spectra (Bruker Ultraflex I) [c)]; stacked MALDI-TOF profile spectra view of BALF samples taken from NSCLC (red) and control (green) patients [d)]; bioinformatic classification of the BALF profile spectra [e)]; mean normalized and smoothed BALF profile spectrum in the mass range below 4 kDa [f)]; box plots of the normalized intensities of three NSCLC biomarker candidate peaks [g)]

Summary

Using our standardized method to acquire MALDI-TOF BALF proteome profile spectra, we have shown the upregulation of histatin 3 and calgranulin C in a small pilot cohort of NSCLC patients. Aside from their potential of becoming clinical biomarkers for lung cancer detection and risk stratification, NSCLC protein biomarkers might also provide new insights into the pathways and mechanisms involved in lung cancer pathogenesis. This pilot study serves to demonstrate that it is feasible to screen a larger NSCLC patient cohort for BALF proteome level biomarkers in a clinical setting.

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