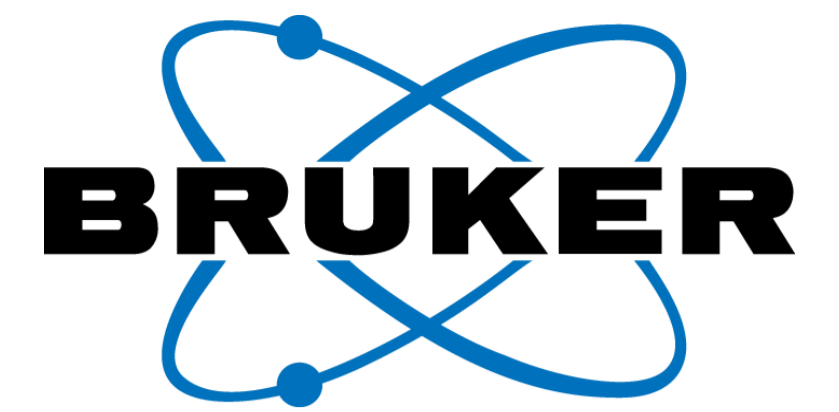


Proteomic Analysis of BALF using the PASEF method : toward Lung Cancer Biomarker Discovery with 1D LC separation



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Full Author List

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Introduction

According to US National Health Center for Health Statistics, the mortality rate of cancer patients decreases every year. When diagnosed at an advanced stage of lung cancer, however patients are expected to have only a ~15% survival rate for 5 years. As such, the need for a method for early diagnosis and treatment of lung cancer has emerged. To solve this problem, we previously performed an in-depth proteomic study using bronchoalveolar lavage fluid (BALF) by combining two dimensional (2D) peptide separation with immuno-depletion of highly abundant proteins in BALF. Here, we performed label-free quantitative proteomic analysis using a high-resolution TIMS-QTOF mass spectrometer to test if one dimensional separation with the depletion protocol would be sensitive and specific enough to find lung cancer biomarkers.

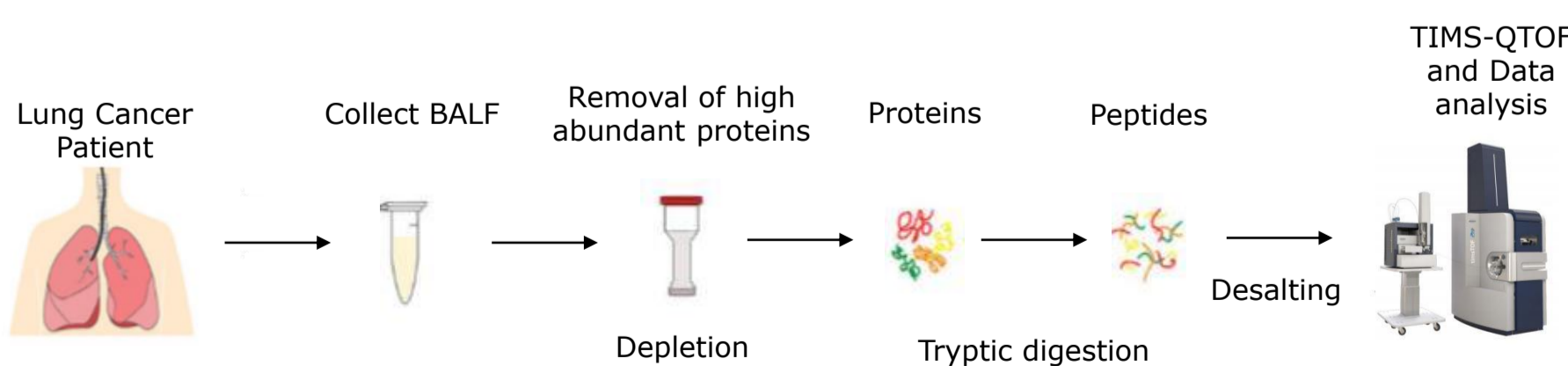


Fig. 1 Experimental workflow employed in this study

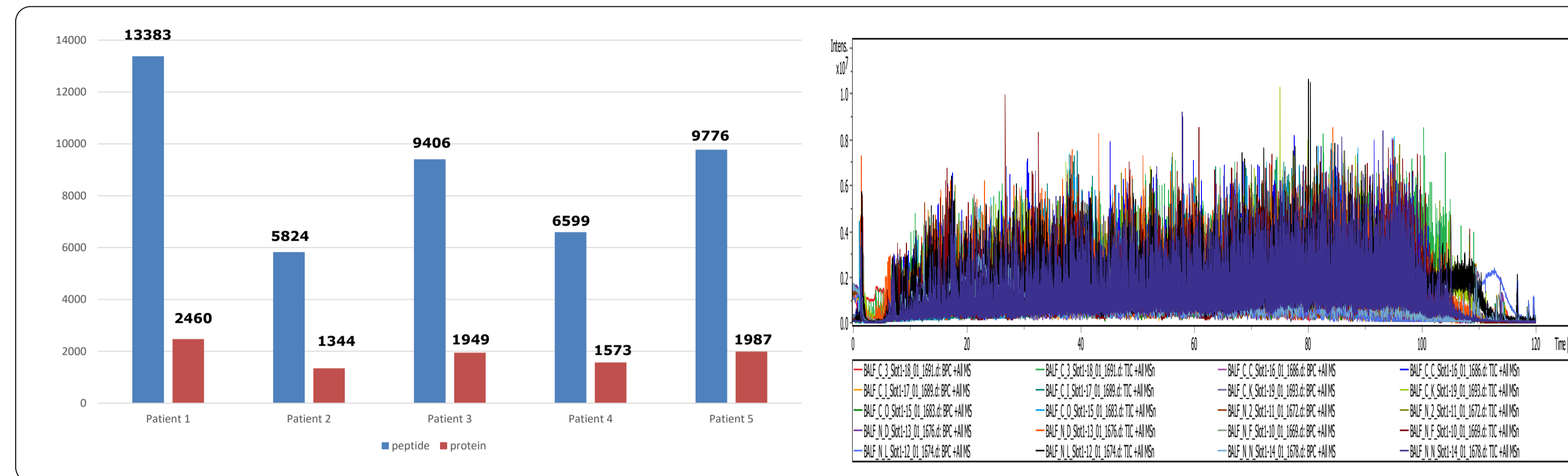


Fig. 2 a) Bar graph of total peptides and proteins matched to each patient, b) Chromatograms of BALF samples from a total of 5 patients. All patients were diagnosed to none small cell lung cancer (NSCLC). Each patient's lung divided two types (cancer/normal).

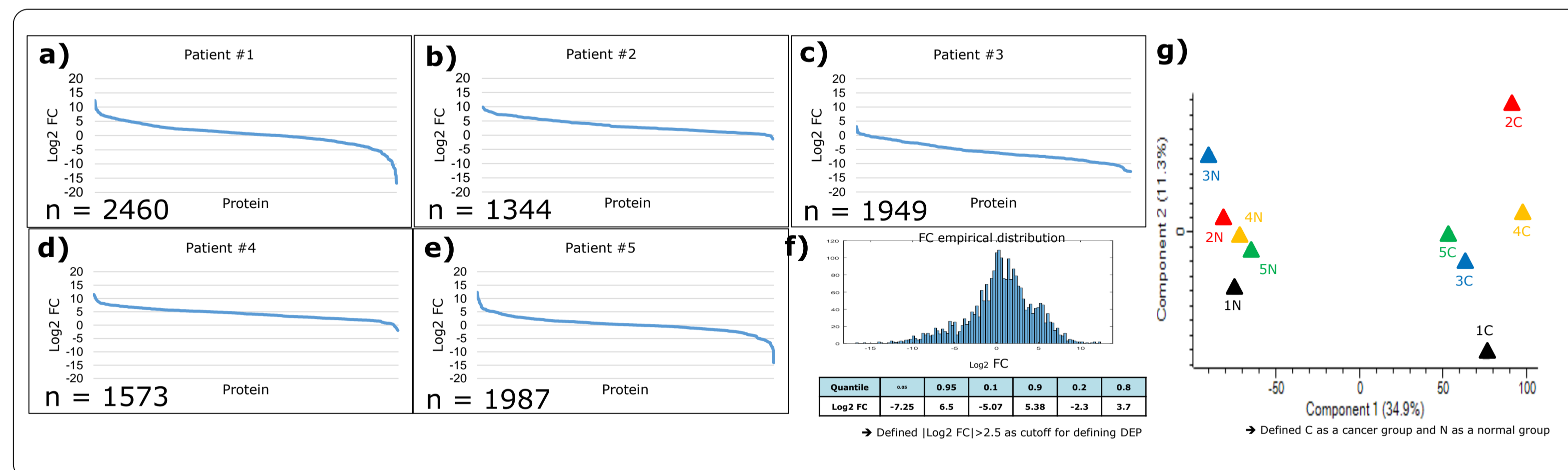


Fig. 3 a) to e) log₂ FC (cancer/normal) value distribution of each patients, f) FC empirical distribution of the entire samples, g) PCA analysis of each samples.

Protein#	Gene	Gene description	Major peptides##
Q9NQR4	NIT2	Omega-amidase NIT2	AVDNQVYVATASPAR; ASYVAWGHSTVNPWGEVLAK; AGTEEAIVYSDIDLK; TSPGDSFSTFDTPYCR
P08567	PLEK	Pleckstrin	EDPAYLHYDPAEADPLGAIHLR; GCVTVSVESNSNGR; QQDHFQAAFLER; SEENLFEIITADEVHYFLQAATPK; GSTLTSPCQDFGKR; IFNHCFGTGNCVIDWLVSNSQSVR
H0Y6E7	H0Y6E7	RNA-binding motif protein, X chromosome (Fragment)	VEQATKPSFESGR; GFAPVTFESPADAK; LFIGGLNLTETNEK

Detected in >=3 patients, positive directionality (Top 3).
Detected in more than half of patient samples.

Table. 1 Table for the Top three DEP proteins. log₂|FC| > 2.5 cutoff value was used for DEP screen and proteins with the same trend were selected in at least three patients.

Methods

Prepared BALF samples were depleted by using High SelectTM Top 14 Abundant Protein Depletion Mini Spin Columns (A36370, Waltham, Massachusetts). Flow-through was collected. Then the protein samples were concentrated using 0.5 mL 3 kDa Amicon filter. After BCA assay, total 5 µg proteins were digested at an enzyme to protein ratio of 1:10 w/w and peptide were desalted using a C18 spin column. After peptide BCA assay, each 100 ng of peptides were dried by using speed vac. All samples store at -80 °C prior to analysis by mass spectrometry. Brief experimental work flow can show the Fig. 1.

The nanoElute HPLC (Bruker Daltonics) was on-line coupled to a high-resolution TIMS-QTOF mass spectrometer with a CapitiveSpray ion Source (timsTOF Pro, Bruker Daltonics). 100 ng of samples were separated on a 250 mm pulled emitter column (IonOpticks, Australia) with 90 min gradient (2-30% ACN), A PASEF cycle of 1.17 s including 1 TIMS MS scan (100ms) and 10 PASEF scans (100 ms each) containing on average 12 MS/MS scans per PASEF scan. Total chromatograms can show Fig. 2. All raw files were analyzed by MaxQuant v1.6.6.0 software using the integrated Andromeda search engine. Experiment type was set as TIMS-DDA and the human Uniprot reference proteome with isoforms (downloaded August 2019) was used as database

Results

A total of 4,075 proteins mapped on 2,144 genes were identified from ten BALF samples, of which 1853 proteins on average were found from a single BALF sample (Fig. 2). When comparing this list of proteins with our previous data, we found that only 1,167 proteins were commonly observed. This indicates lungs of each patient may have reflect variation based on their individual genetic background and a collection of environmental factors. To see how diverse BALF proteomes between patients are, we analyzed this dataset by comparing identified proteins per patient or per disease state. Subsequently, fold change (FC) was calculated for each cancer-normal pair per patient and a cutoff of log₂ FC > 2.5 was used to define differential expression (Fig. 3). Differentially expressed proteins (DEPs) for at least 3 cancer-normal pairs were identified and highly upregulated proteins in cancer BALFs as compared to normal BALFs were listed up as potential BALF biomarkers (Table. 1).

Conclusion

Among these potential biomarkers were protein products of NIT2 and PLEK. Firstly, omega-amidase NIT2 encoded by NIT2 gene has been reported to associate with human malignancies including colon cancers. But its expression in lung cancer has not been described thoroughly. Secondly pleckstrin encoded by PLEK gene is a major protein kinase C substrate of platelets. Pleckstrin has been known as a marker for megakaryocytes whose abundance were found to be increased in pulmonary artery blood in lung cancer patients. In conclusion, a sensitive label-free quantitative proteomics of BALF using timsTOF Pro can help to identify biomarkers for lung cancers.

References

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