

# Application Note: Tissue Proteomics In Formalin Fixed Tissue Using Mass Spectrometry

## Identification of Prognostic Biomarkers for Prediction of Patient Survival in Metastatic Lung Cancer

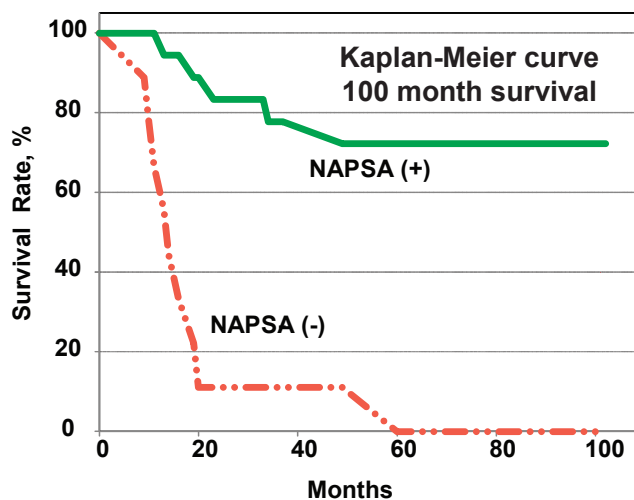
**S**urgery is the first line of therapy for primary lung cancer, which is then followed up by radiation and/or chemotherapy. After removal of the primary tumor, a significant proportion of patients undergoing resection manifest evidence of non-detectable metastatic disease and show low survival rates. Recently, high resolution mass spectrometry-based proteomics technologies have evolved to enable expression analysis of proteins from formalin-fixed, paraffin-embedded tissue samples (FFPE).

A research group from Tokyo Medical University and Biosys Technologies, Inc. (Japan), headed by Professor Toshihide Nishimura has utilized a suite of tissue microproteomics technologies (Figure 3) to evaluate capabilities for discovering protein biomarkers of metastatic lung cancer directly in formalin-fixed archival lung cancer tissue. Analysis of these tissue specimens from patients with divergent clinical courses identifies novel prognostic protein biomarkers that better diagnose the stage of lung cancer and, furthermore, can correlate these proteins against survival data to predict patient survival post-surgery (Figure 1).

### Methods

Archived formalin-fixed sections of primary stage IA lung cancer, primary stage IIIA lung cancer, and immediate surrounding lymph nodes containing metastatic IIIA lung cancer were obtained from patients who underwent surgical resections. Many of these patients developed recurrent disease and passed away within 3 years, whereas others remained disease-free for at least 3 years post-surgery.

Figure 1: MRM quantitation indicates that NapsinA is capable of predicting lung cancer survival.



Cancerous epithelial cells were obtained by tissue microdissection on a Leica LMD6000™ instrument (Leica Microsystems) utilizing Director® slides (Expression Pathology Inc.). Proteins were extracted and prepared using Liquid Tissue® reagents and protocol (Expression Pathology Inc.), and then analyzed by global high resolution tandem mass spectrometry on a ZAPLOUS MDLC-MS chromatography system (AMR Inc.) coupled to an LTQ mass spectrometer (Thermo Scientific). Ion intensity levels were determined by Expressionist® Refiner MS software (Genedata) to provide relative quantitation to search for differentially expressed proteins between three (3) stage IA primary tumor, and three (3) stage IIIA primary tumor that showed metastasis to the mediastinal lymph nodes, and the cancerous epithelium from a mediastinal lymph node containing stage IIIA metastatic disease.

Liquid Tissue® preparations from multiple samples of each stage were then interrogated by SRM and MRM analyses utilizing a 4000 Q Trap® triple quad instrument (Applied Biosystems/MDS Sciex) in order to confirm and validate candidate biomarkers of metastatic lung cancer and to investigate their use for prediction of survival. The relative quantitation of individual proteins was normalized to both a pool of all the samples interrogated in this study as well as against a handful of housekeeping proteins providing for an In-Sample internal standard.

## Results

Over 600 unique proteins were identified from the primary global expression profiling between stage IA and stage IIIA metastatic lung cancer (Figure 2). Statistical analysis identified 234 commonly expressed proteins and 404 differentially expressed peptides between the stage IA and the stage IIIA samples. An additional global profile uncovered over 371 proteins differentially expressed between stage IIIA primary tumors and metastatic epithelial cells procured from disease mediastinal lymph nodes. Based on the degree of difference and the potential biological relevance of each individual candidate biomarker, a total of 14 proteins were subjected to follow-up MRM analysis for further evaluation. In-depth analysis indicates the ability to provide relative quantitative measure of these 14 proteins across many tissue samples that derive from both stage IA and stage IIIA primary tumors, as well as across multiple cases of immediate surrounding lymph nodes harboring metastatic stage IIIA disease. MRM analyses of these cases further narrowed the candidate biomarkers down to a single protein that may be predictive to 3 year survival rates for primary tumors of stage IA lung cancer (Figure 3).

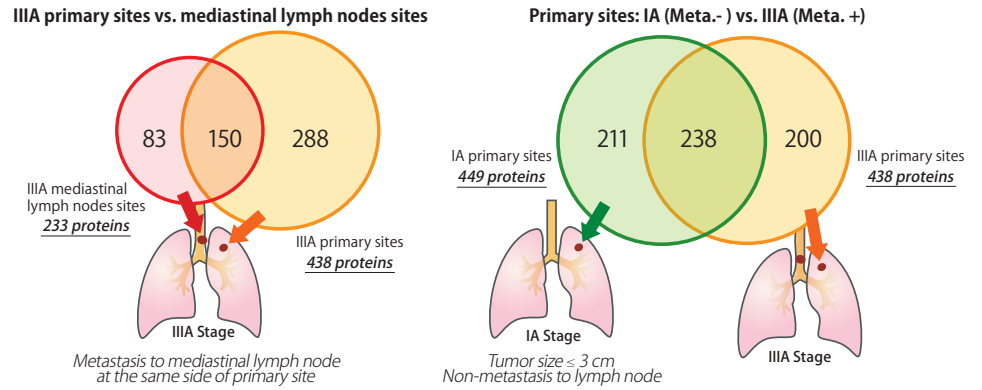


Figure 2: Summary of Global Protein Expression Profiling of Primary Lung Cancer Tissue.

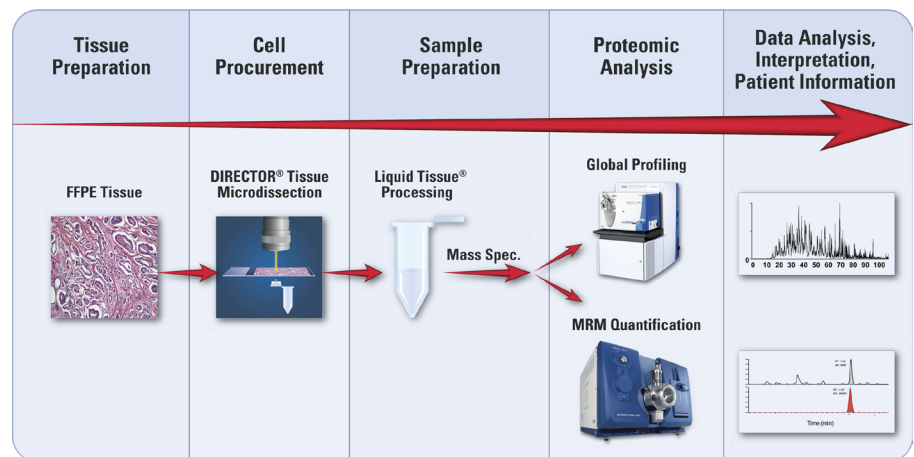


Figure 3: Platform for proteomic analysis of protein biomarkers in formalin-fixed paraffin-embedded tissue using mass spectrometry.

## Conclusion

This novel combination of technologies has enabled proteomic discovery and analysis of previously unusable archived formalin-fixed paraffin-embedded tissue, first in a semi-quantitative manner, then in a more targeted way to focus on specific individual proteins. Further studies are now under way to assess the utility of these candidate proteins as indicators of the stage of disease and predictors of disease recurrence and time of survival.

Data courtesy  
 Professor Toshihide Nishimura, Tokyo Medical University, and  
 Mr. Yasuhiko Bando, Biosys Technologies, Inc.

Liquid Tissue technology is protected by U.S. Patent 7,473,532 and patents pending and foreign equivalents thereof.

DIRECTOR technology is protected by U.S. Patents 7,294,367 and 7,381,440 and foreign equivalents thereof.

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