Scaling Discovery Proteomics to Large Lung Cancer Cohorts using Data Independent Acquisition

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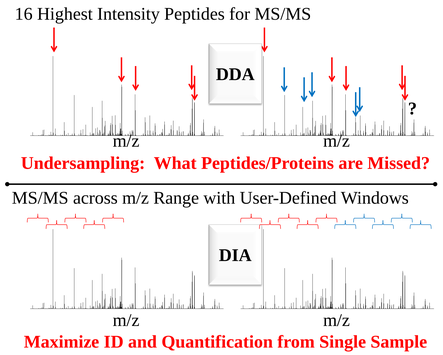
Introduction

Proteome profiling experiments must satisfy two primary requirements: maximizing depth of protein sampling as well as scaling to clinical cohorts. The approach presented here integrates discovery experiments used to create reference databases and rapid data independent acquisition (DIA) methods to examine large clinical cohorts using < 3 hours of instrument time per individual patient's resected tumor specimen.[1-2] The combination facilitates data mining for protein identification and relative quantitation across larger sample sets within 1 month.

DIA LC-MS/MS proteome profiling data were acquired from lung squamous cell carcinoma, adenocarcinoma, and adjacent lung tissue. DIA analysis of cores from a tissue microarray produced quantitative data for > 3,000 proteins each, indicating that the technology can be applied to minimal amounts of formalin fixed paraffin embedded tumor tissue.



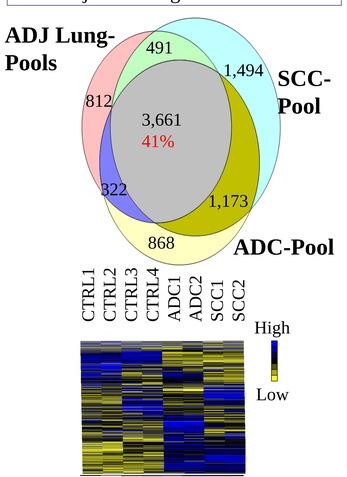
Experimental Details

Sample Preparation: FFPE Tissue was processed using the method reported by Sprung *et a* l[3]. Twelve fractions from basic pH reversed phased liquid chromatography (bRPLC) were analyzed using LC-MS/MS. Unfractionated digests were analyzed using pSMART and hybrid DIA acquisition methods.

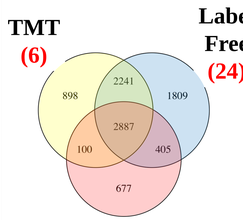
Spectral Library Construction: Sequest and Mascot searches were used for spectral library generation (Proteome Discoverer 1.4, Thermo Fisher Scientific).

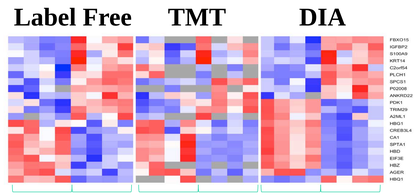
DIA and Data Processing: Samples were analyzed with MS[1] followed by narrow window DIA (n = 18) in each cycle. The data were matched to the spectral libraries and qualitative and quantitative data processing was performed using Pinnacle (OPTYS Tech). In addition, hybrid acquisition with DIA and targeted MS[2] is examined using cancer biomarkers.

Lung Tissue Sample Type	#
Squamous Cell Carcinoma (SCC)	50
SCC Adjacent Lung Tissue	50
Adenocarcinoma (ADC)	46
ADC Adjacent Lung Tissue	13

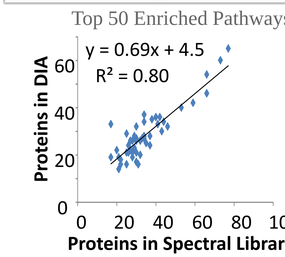


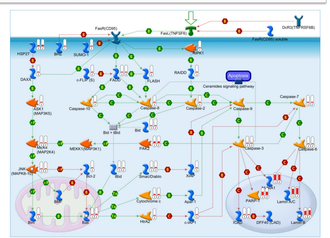
Comparison of Discovery Methods





Pilot Project Comparing Squamous Tumors to Adjacent Lung Tissue using Label-Free, TMT, and DIA Discovery Proteomics Enables Investigation of Detectable Biology.

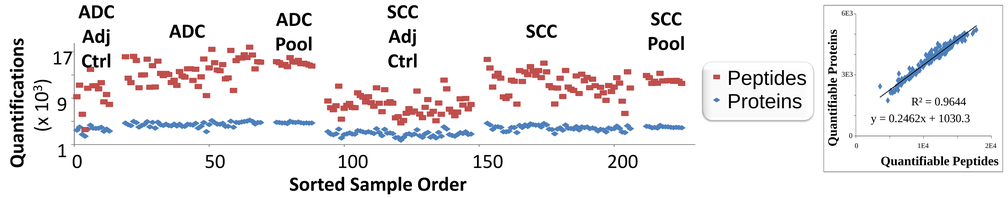




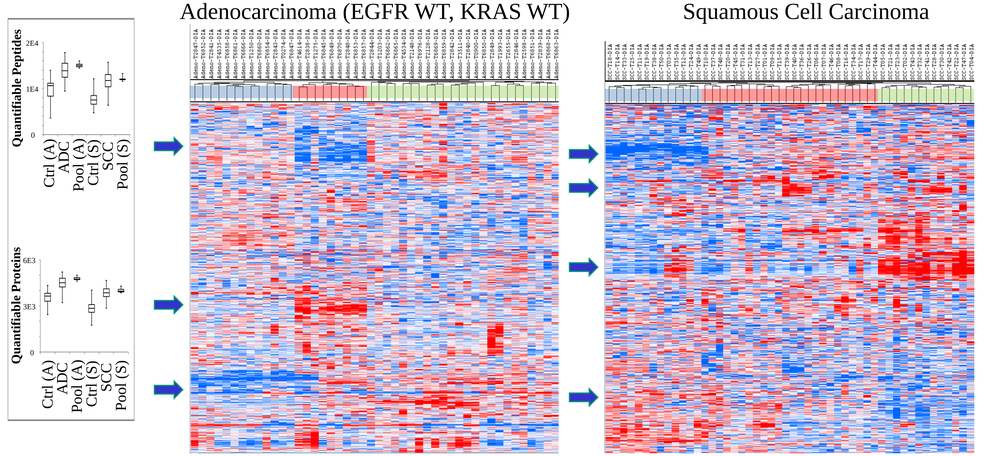
Apoptosis Signaling, GeneGO, Metacore

Selected Cancer-Related Proteins Not Observed in DIA, but in Spectral Library: 4E-BP1, AKT(PKB), ASK1 (MAP3K5), Bcl-2, Bcl-XL, Bcl-XS, Bim, Collagen IV, COX-2 (PTGS2), c-Src, Frizzled, GIPC, GSK3 alpha/beta, GYS1, HGF receptor (Met), IBP, IGF-1 receptor, IGF-2, I-kB, IKK-beta, IKK-gamma, Insulin receptor, IRAK1/2, IRAK4, K-RAS, Matrilysin (MMP-7), MEK1/2, MEK3(MAP2K3), MEK6(MAP2K6), MELC, Midkine, MMP-1, MNK1, MRLC, MyD88, Neurofibromin, NF-κB, N-WASP, p130CAS, PARD6, PDK (PDPK1), PI3K cat class IA, PI3K cat class IA (p110-alpha), PI3K cat class IB (p110-gamma), PI3K reg class IA, PI3K reg class IA (p85), PI3K reg class IA (p85-alpha), PI3K reg class IA (p85-beta), PKC-zeta, PLAUR (uPAR), PP2A catalytic, PTEN, RHEB2, Shc, SMAD2, SP1, TAB1, Talin, TGF-beta 1, Tryptase, VCAM1, VEGF-A, WNT, XIAP

Molecular Classification using Lung Cancer Proteomes

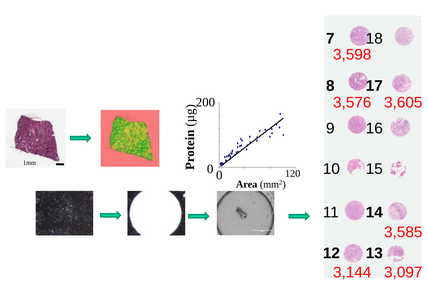


Assessment of 159 Total Tumor and Lung Tissue Proteomes using 200 DIA-LC-MS/MS Analyses over 25 Days Enables Molecular Classification of Tumors.



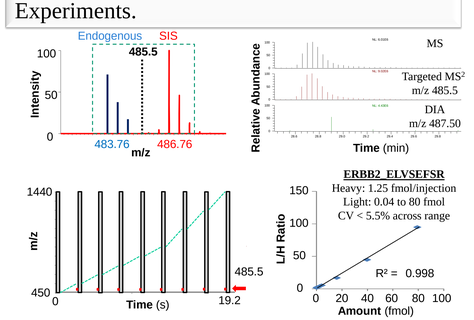
Tissue Microarray Analysis

Protein Quantification from Single TMA Core Sections Builds on Whole Slide Imaging (Aperio), Histological Maps (Genie) , and DIA-LC-MS/MS Analysis.



Targeted Quantification in DIA

Targeted Peptide-Based Quantitative Assays for Known Biomarkers (*e.g.* HER2) can be Built into DIA Proteome Analysis Experiments.



Conclusions

1. DIA balances a sufficient depth of coverage with the ability to analyze large cohorts of patients (n = 100-400) within 1-2 months on a single instrument.
2. Hybrid data acquisition / processing method developed, which includes spectral library generation, scans combining MS[1], asymmetric narrow window DIA, targeted MS[2] scans, spectral library matching and dual MS[1]/MS[2] quantitation using Pinnacle software.
3. Applicable to Tumor Microarray Specimens.

References

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3. Sprung RW Jr, *et al. Mol Cell Proteomics* **2009**, *8*, 1988-98.
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Acknowledgments

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