Glycoproteomic analysis of formalin-fixed and paraffin-embedded tissues and OCT-embedded frozen tissues

Yuan Tian, Kay Gurley, Steve G. Bova, Christopher J. Kemp, and Hui Zhang

Introduction

- FFPE tissues and OCT-embedded tissues are commonly used and archived with pathological and clinical information;

- Applications:
  - Histology
  - Immunohistochemistry for molecular marker
  - In situ hybridization
  - Proteomics

FFPE: Formalin- **F**ixed **P**araffin-**E**mbedded
OCT: Optimal **C**utting **T**emperature compound

Introduction

Johns Hopkins Medicine

Glycoproteins as Serum Tumor Markers

- Prevalent in extracellular proteins
- Most likely detected as surrogate markers
- Altered glycosylation associated with cancer
- All FDA approved protein serum markers are glycoproteins

Introduction

- ❖ Can these processed tissues be used for proteomics analysis by mass spectrometer?

  OCT compound consists of a resin-polyvinyl alcohol, benzalonium chloride, an antifungal agent, and **polyethylene glycol** to lower the freezing temperature

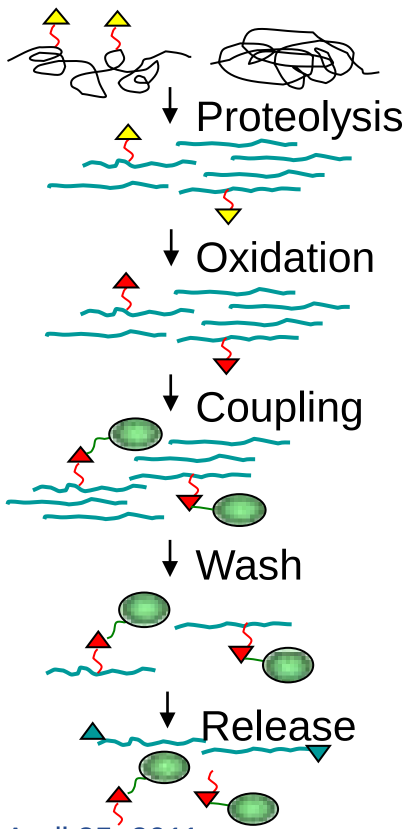
- ❖ Glycosylation is preserved in the embedded tissues?

Aim

- To investigate the feasibility of glycoproteomic analysis using FFPE tissue and OCT-embedded frozen tissue

Method

Solid phase extraction of glycopeptides (SPEG) and quantitative glycoproteomics

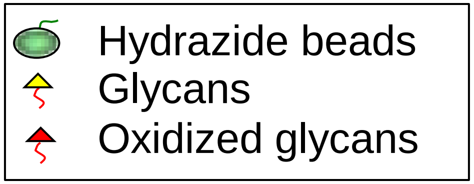


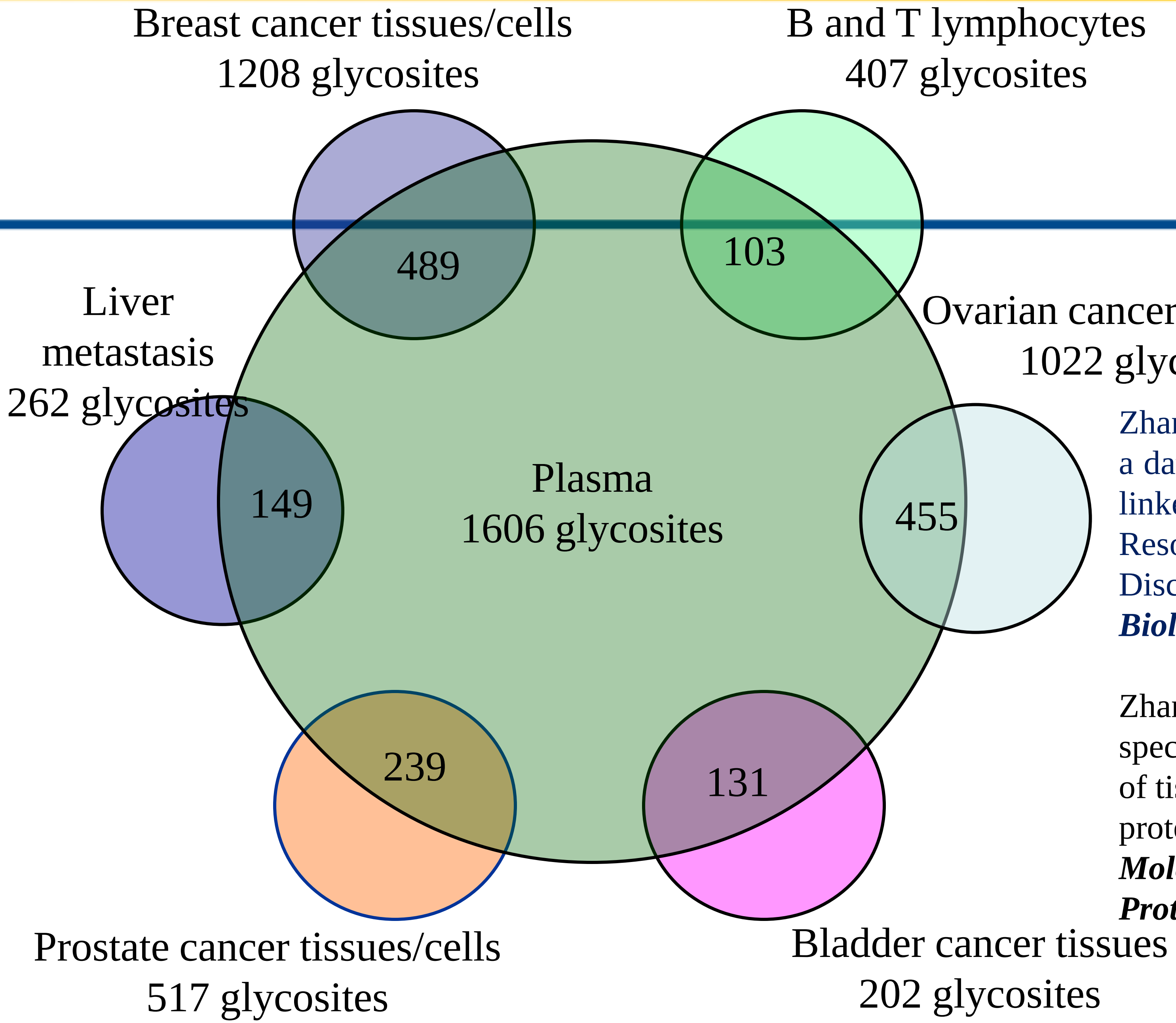
Zhang, H. Li, X-J, Martin, D. M., and Aebersold, R. ***Nature Biotechnology*** **(2003)**.

Tian, Y., Zhou, Y., Elliott, S., Aebersold, R., and Zhang. H. ***Nature Protocols (2007)***.

Zhou, Y. et al. Isolation of *N*-linked glycopeptides from plasma. ***Analytical Chemistry (2007).***

Zou, Z., Ibisate, M., Zhou, Y., Aebersold, R. *Xia*, Y., and Zhang, H. Synthesis and Evaluation of Superparamagnetic Nanoparticles for Extraction of Glycopeptides in the Microtiter Plate Format ***Analytical Chemistry (2008).***



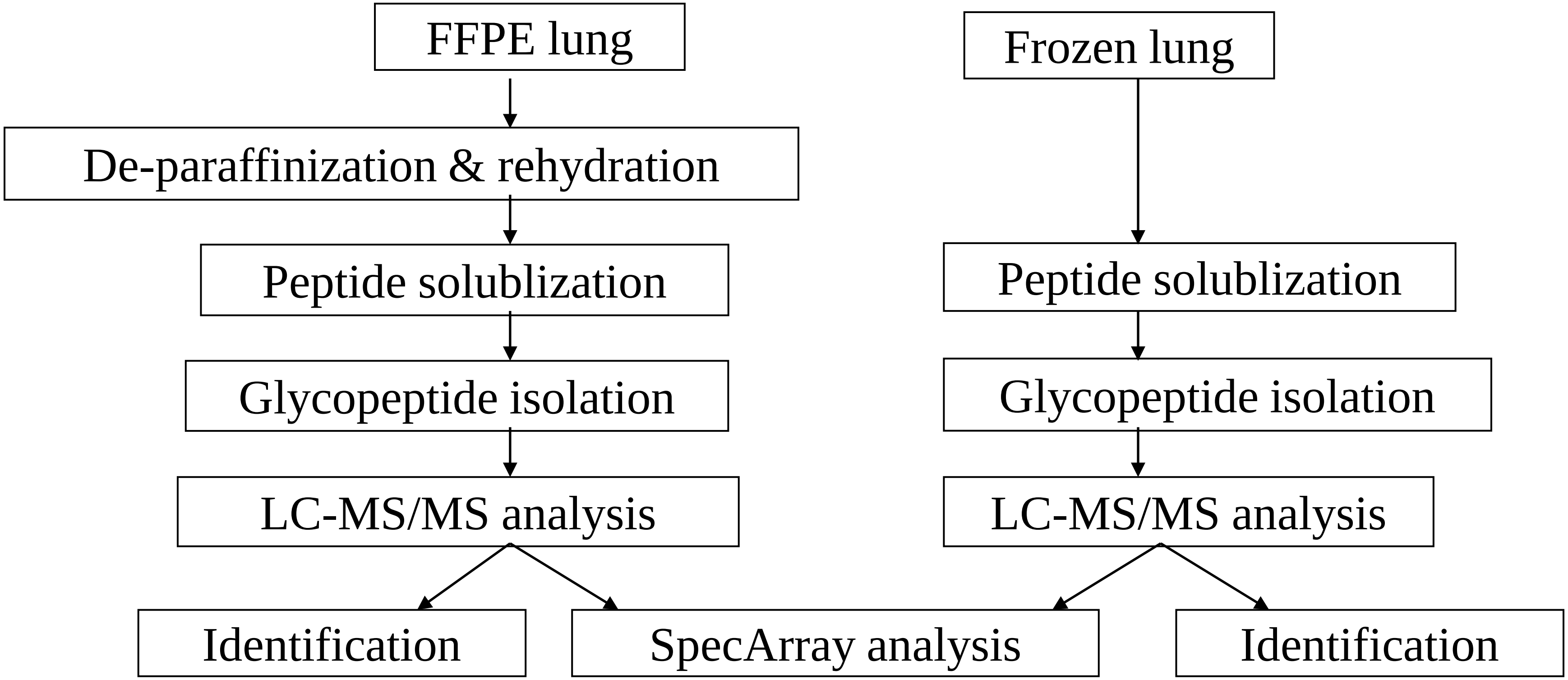


Zhang, H. et al. UniPep, a database for human *N*-linked glycosites: A Resource for Biomarker Discovery. ***Genome Biology* (2006)**.

Zhang, H. et al. Mass spectrometric detection of tissue-derived proteins in blood. ***Molecular & Cellular Proteomics (2007)***

Studies

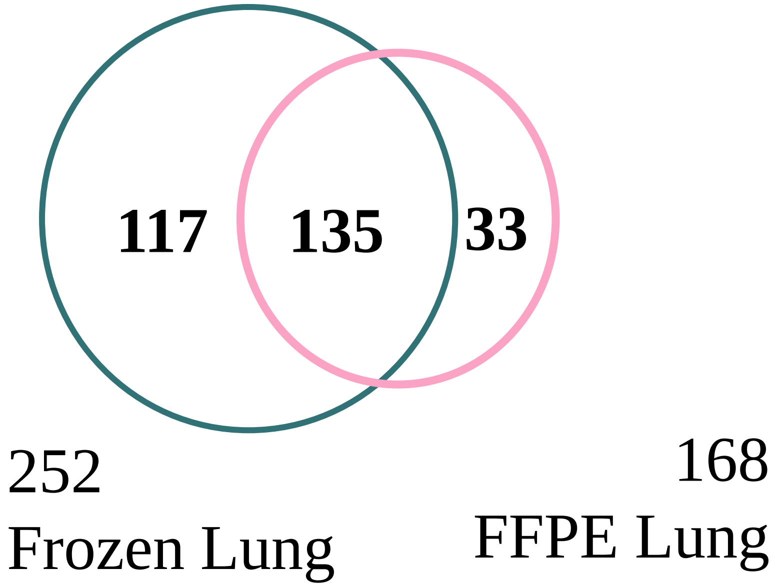
I. Glycoprotein analysis of FFPE tissue vs. frozen tissue



Studies

I. Glycoprotein analysis of FFPE tissue vs. frozen tissue

1. Unique glycosylation sites



Tian Y. et al *J Proteome Res* 2009, 8 (4), 1657-1662

Studies

I. Glycoprotein analysis of FFPE tissue vs. frozen tissue

2. Tryptic ends & missed cleavage sites

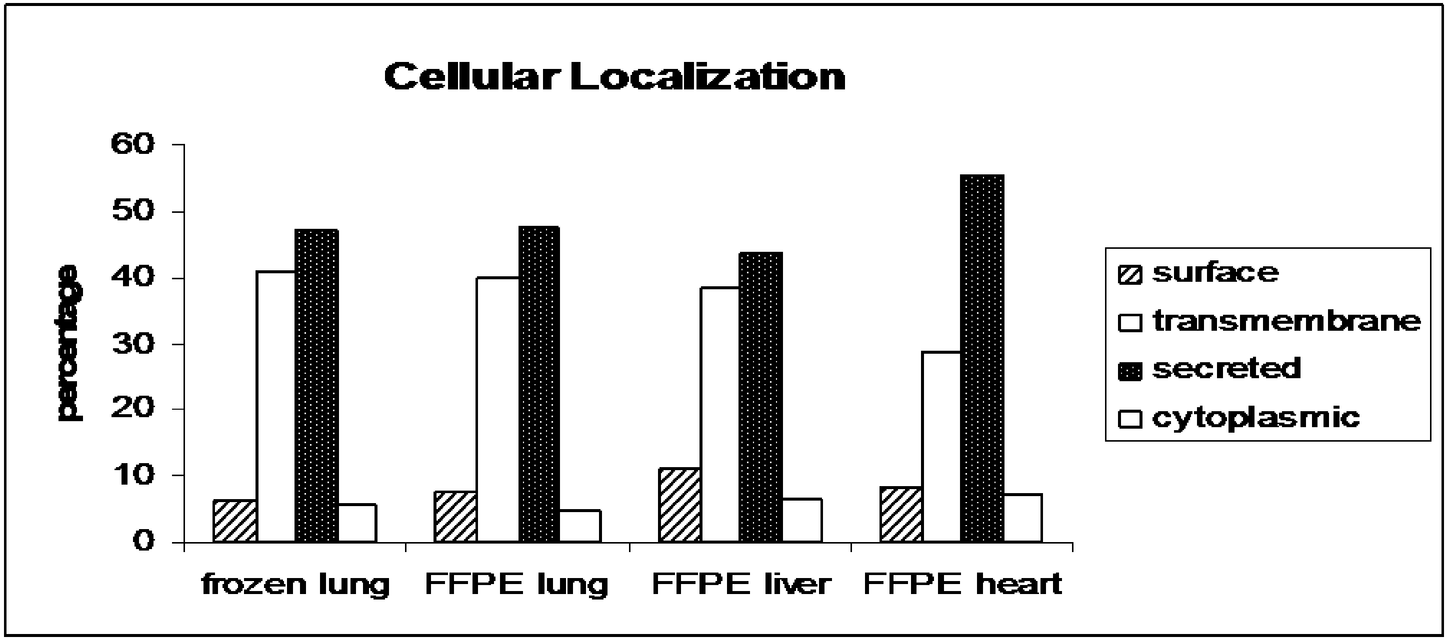
	% of 1 tryptic end	% of 2 tryptic end	% of -R	% of -K	missed cleavage site %
FFPE lung	63.10	36.9	51.63	48.37	16.07
Frozen lung	64.68	35.32	50.43	49.57	17.06

Tian Y. et al *J Proteome Res* 2009, 8 (4), 1657-1662

Studies

I. Glycoprotein analysis of FFPE tissue vs. frozen tissue

3. Cellular location of identified glycoproteins

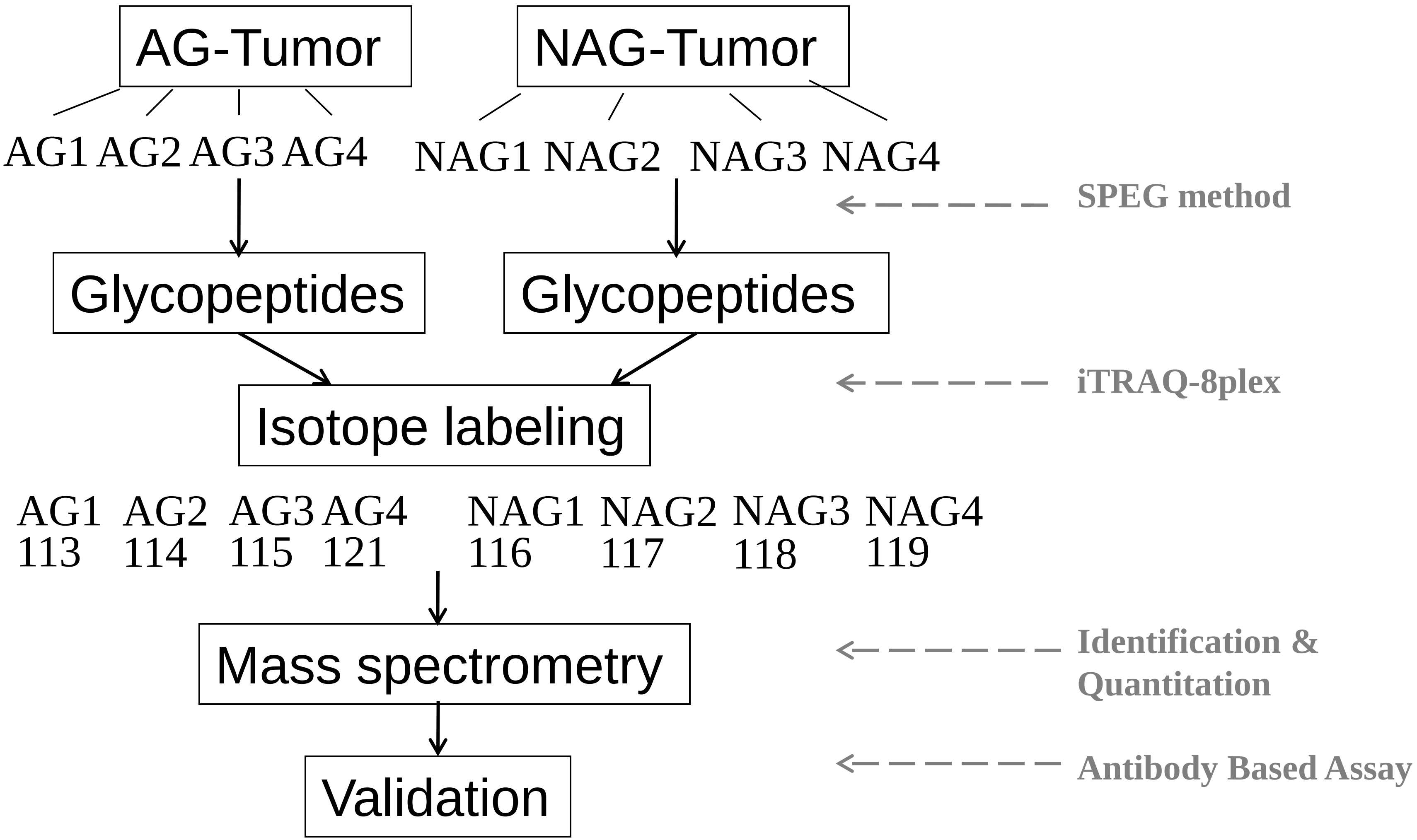


Tian Y. et al *J Proteome Res* 2009, 8 (4), 1657-1662

Studies

II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues

- Prostate cancer is the most common malignancy in men in the United States;
- No good method to reliably distinguish aggressive from non-aggressive prostate cancer

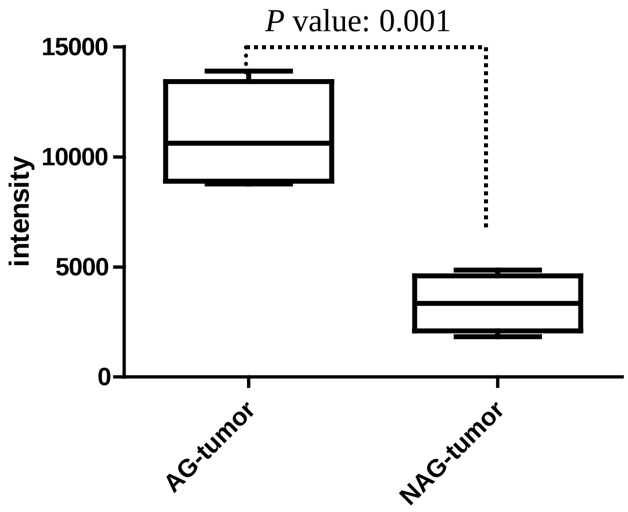

AG-Tumor
NAG-Tumor
AG1 AG2 AG3 AG4
NAG1 NAG2 NAG3 NAG4
SPEG method
Glycopeptides
Glycopeptides
iTRAQ-8plex
Isotope labeling
AG1 113
AG2 114
AG3 115
AG4 121
NAG1 116
NAG2 117
NAG3 118
NAG4 119
Mass spectrometry
Identification & Quantitation
Validation
Antibody Based Assay

Studies

II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues

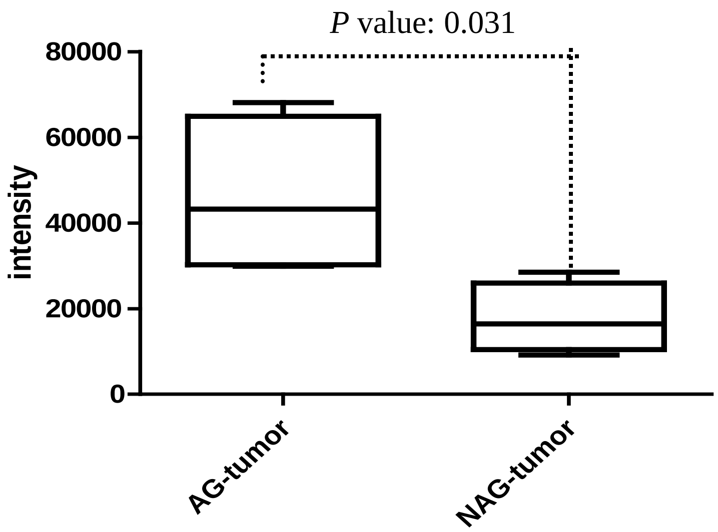
A

microfibrillar-associated protein 4_pep1



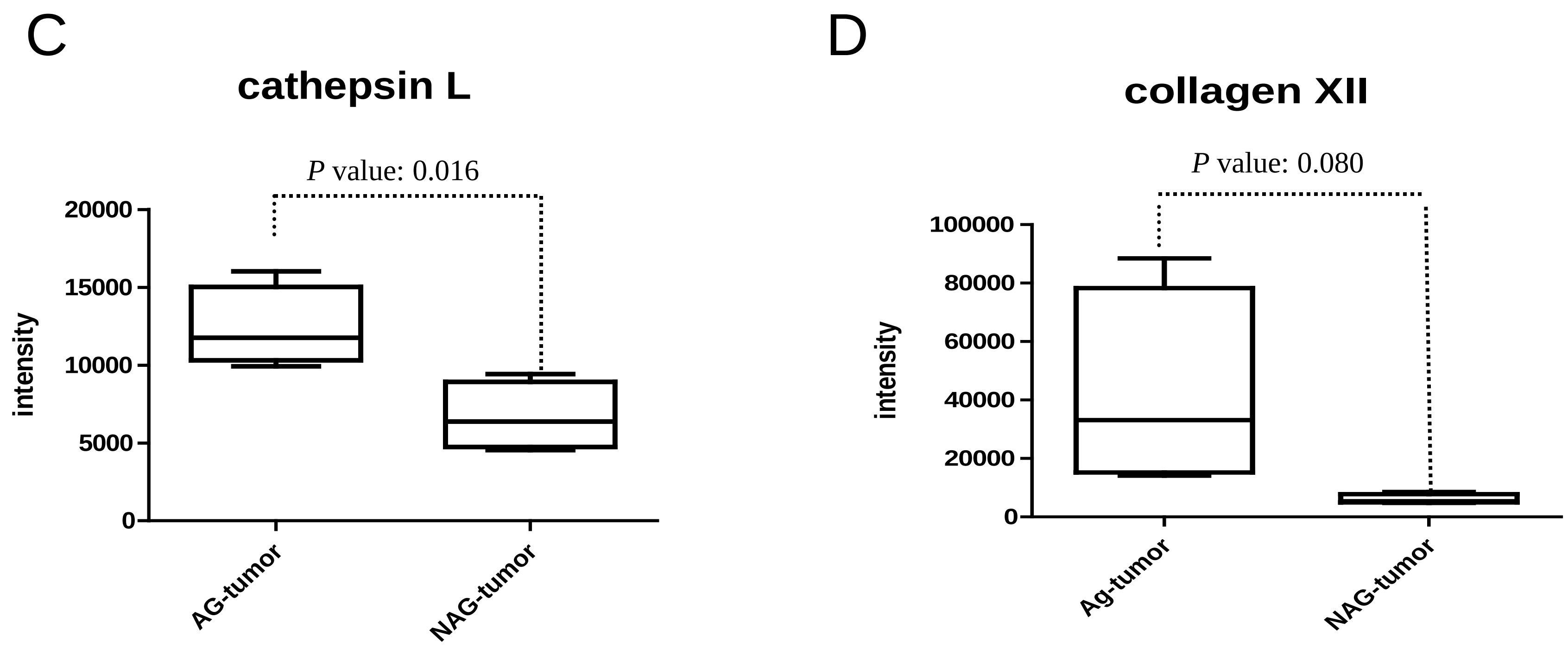
B

periostin



Studies

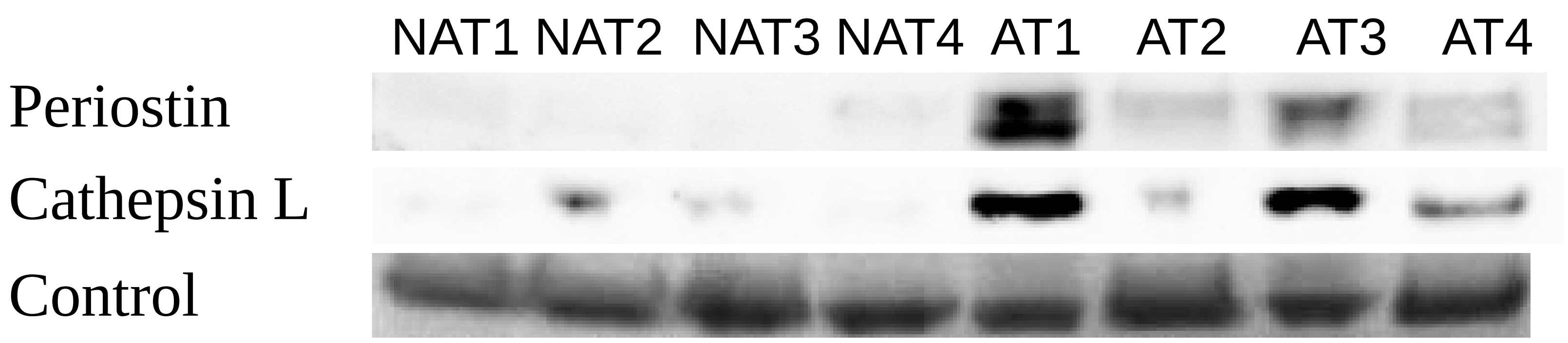
II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues



Studies

II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues

Verification using Western blot



Studies

II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues

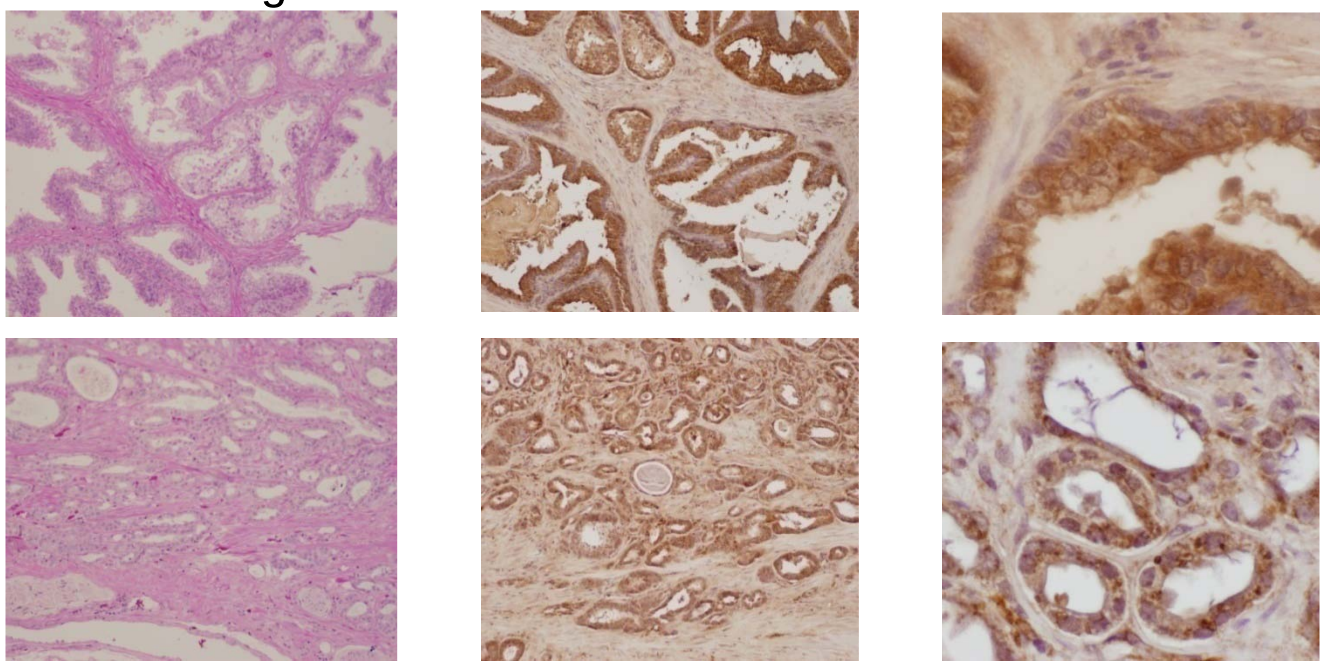
Verification using immunohistochemistry

Cathepsin L

H&E staining | 100X | 600X

Normal

Primary prostate cancer



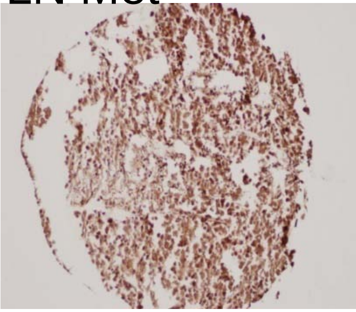
Studies

II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues

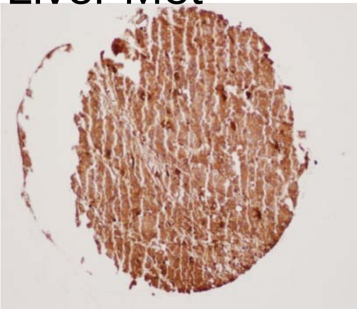
Verification using immunohistochemistry

Cathepsin L

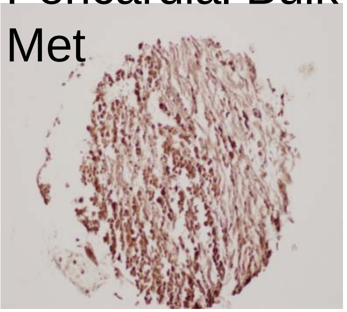
LN Met



Liver Met

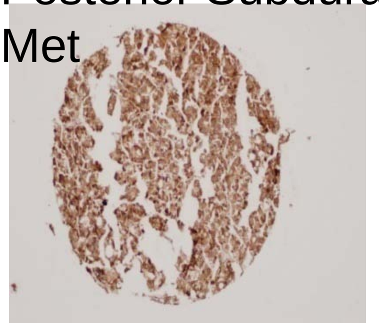


Pericardial Bulk Met

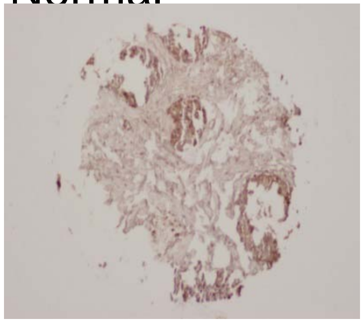


Spleen Met

Posterior Subdura Met



Normal



Studies

II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues

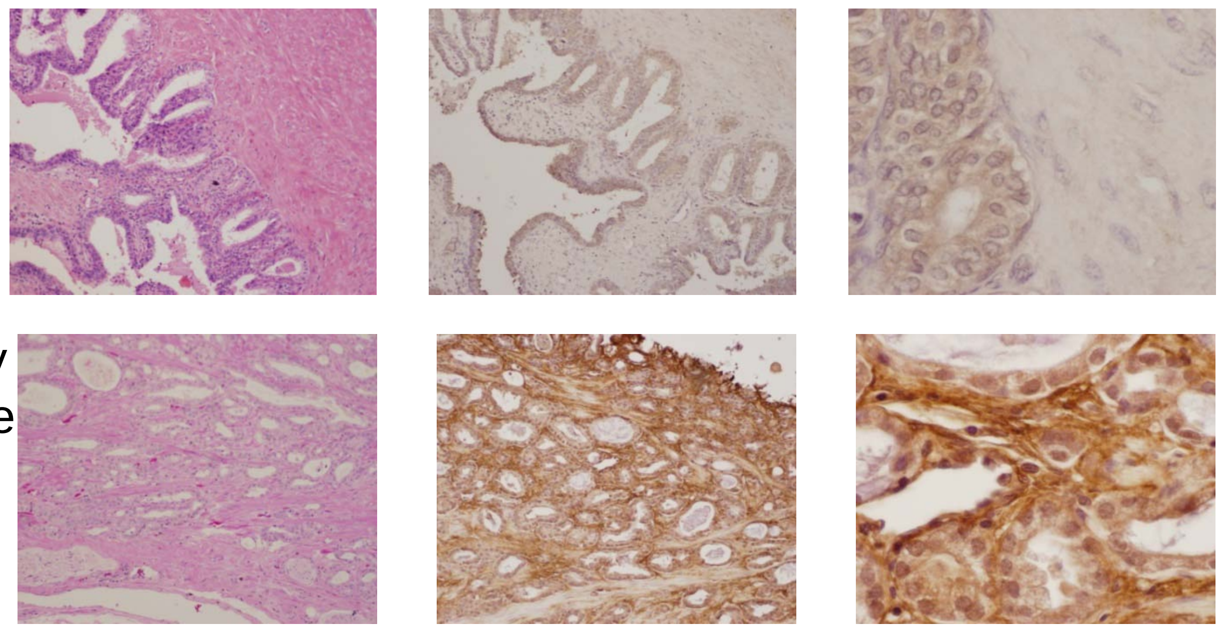
Verification using immunohistochemistry

Periostin

H&E 100X 600X

Normal

Primary prostate cancer

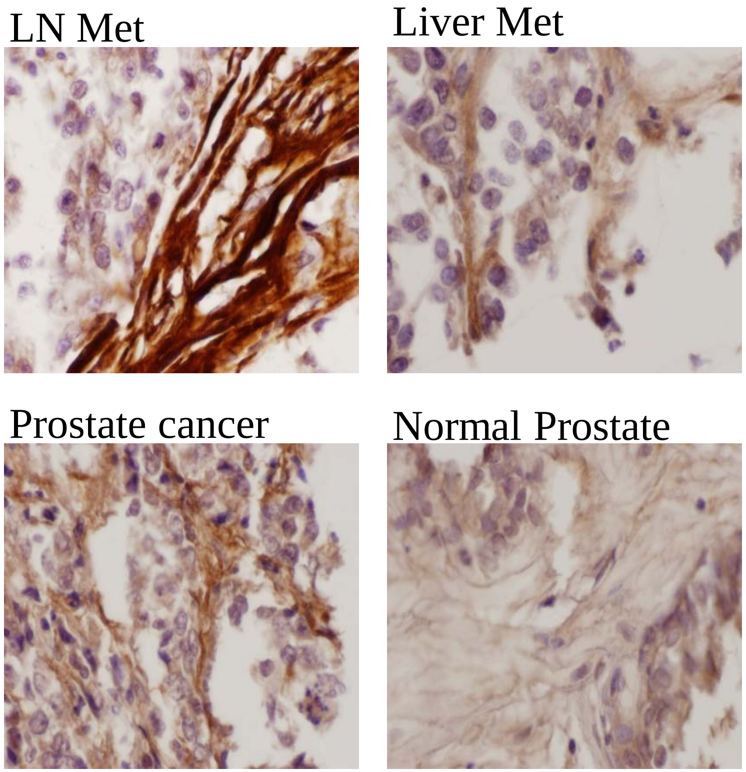


Studies

II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues

Verification using immunohistochemistry

Periostin



JOHNS HOPKINS MEDICINE

Conclusion

- FFPE tissues and OCT-embedded frozen tissues are feasible to be used in glycoproteomics analysis
- Glycans can be used as tags to isolate glycoproteins from FFPE and OCT-embedded biospecimens

Acknowledgements

Johns Hopkins University

Hui Zhang

Daniel Chan

Xiaer Sun

G. Steve Bova

Dylan Bach

Robert N. Cole

Robert O'Meally

Fred Hutchinson Cancer Research Center

Christopher J. Kemp

Kay Gurley

Grant support

NIH/NCI

Early Detection Research Network (EDRN)

Patrick C. Walsh Prostate Cancer Research Fund