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- Fixed Paraffin-Embedded

**OCT**: Optimal Cutting Temperature compound
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


Breast cancer tissues/cells
1208 glycosites

B and T lymphocytes
407 glycosites

Liver metastasis
262 glycosites

Ovarian cancer tissues/cells
1022 glycosites

Prostate cancer tissues/cells
517 glycosites

Bladder cancer tissues
202 glycosites

Plasma
1606 glycosites


I. Glycoprotein analysis of FFPE tissue vs. frozen tissue

FFPE lung
- De-paraffinization & rehydration
- Peptide solubilization
- Glycopeptide isolation
- LC-MS/MS analysis
  - Identification
  - SpecArray analysis

Frozen lung
- Peptide solubilization
- Glycopeptide isolation
- LC-MS/MS analysis
  - Identification
Studies

I. Glycoprotein analysis of FFPE tissue vs. frozen tissue

1. Unique glycosylation sites

<table>
<thead>
<tr>
<th>Frozen Lung</th>
<th>FFPE Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>252</td>
<td>168</td>
</tr>
<tr>
<td>117</td>
<td>135</td>
</tr>
<tr>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

Tian Y. et al *J Proteome Res* 2009, 8 (4), 1657-1662
I. Glycoprotein analysis of FFPE tissue vs. frozen tissue

2. Tryptic ends & missed cleavage sites

<table>
<thead>
<tr>
<th></th>
<th>% of 1 tryptic end</th>
<th>% of 2 tryptic end</th>
<th>% of -R</th>
<th>% of -K</th>
<th>missed cleavage site %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFPE lung</td>
<td>63.10</td>
<td>36.9</td>
<td>51.63</td>
<td>48.37</td>
<td>16.07</td>
</tr>
<tr>
<td>Frozen lung</td>
<td>64.68</td>
<td>35.32</td>
<td>50.43</td>
<td>49.57</td>
<td>17.06</td>
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</tbody>
</table>

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

AG1 AG2 AG3 AG4

Glycopeptides

Isotope labeling

AG1 AG2 AG3 AG4
113 114 115 121

NAG-Tumor

NAG1 NAG2 NAG3 NAG4

Glycopeptides

Isotope labeling

NAG1 NAG2 NAG3 NAG4
116 117 118 119

Mass spectrometry

Validation

SPEG method

iTRAQ-8plex

Identification & Quantitation

Antibody Based Assay
Studie

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

A

microfibrillar-associated protein 4_pep1

B

periostin

\[ P \text{ value: 0.001} \]

\[ P \text{ value: 0.031} \]
II. Identification of glycoproteins associated with aggressive prostate cancer using OCT-embedded tissues

C. **cathepsin L**

D. **collagen XII**

\[ P \text{ value: 0.016} \]

\[ P \text{ value: 0.080} \]
Studies

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

Verification using Western blot

<table>
<thead>
<tr>
<th>Periostin</th>
<th>Cathepsin L</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT1</td>
<td>NAT2</td>
<td>NAT3</td>
</tr>
<tr>
<td>NAT4</td>
<td>AT1</td>
<td>AT2</td>
</tr>
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Studies

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

Verification using immunohistochemistry

<table>
<thead>
<tr>
<th>Cathepsin L</th>
<th>H&amp;E staining</th>
<th>100X</th>
<th>600X</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
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<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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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

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

Verification using immunohistochemistry

Periostin
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
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