Protein analysis of glioblastoma formalin-fixed paraffin-embedded tissues

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Introduction

- Glioblastoma (GBM) is the most common and malignant brain tumor in adults and has a poor prognosis with a median survival of 15 months.
- Protein analysis of GBM tissues is urgently needed to identify potential protein biomarkers that may assist in early disease diagnosis and therapy development.
- However, simple and reliable protocols that can be implemented in clinics for proteome detection from minute tissue sections remain lacking.
- Here, we report methods for protein analysis from small quantities (~1 mm^2) of GBM formalin-fixed paraffin-embedded (FFPE) tissue sections.

Methods

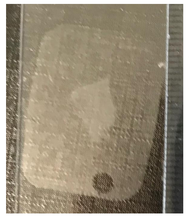

Figure 1. Microdissection of the GBM FFPE tissue.

GBM FFPE tissue blocks

4 µm | 10 µm | 15 µm | 20 µm

Deparafinization in xylene and hydration through an EtOH gradient

8 M urea/30% ACN /100 mM NH_4HCO_3

30% ACN/100 mM NH_4HCO_3

0.1% DDM/8 M urea/30% ACN /100 mM NH_4HCO_3

0.1% RapiGest/8 M urea/30% ACN /100 mM NH_4HCO_3

Overnight trypsin digestion

1 mm^2 injected for LC-MS/MS analysis on a TripleTOF 5600+ (AB Sciex) instrument

Results

Comparison of extraction buffers in identified protein groups, identified peptides, and acquired spectra

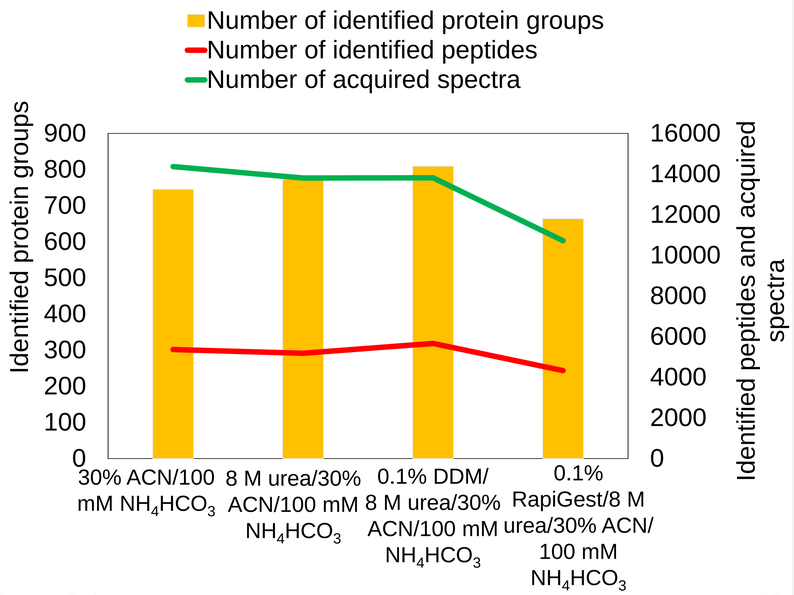

Figure 2. Protein identifications, identified peptides and acquired spectra for 15 µm thick GBM FFPE tissue using the different extraction buffers.

Overlap in identified protein groups between the different extraction buffers

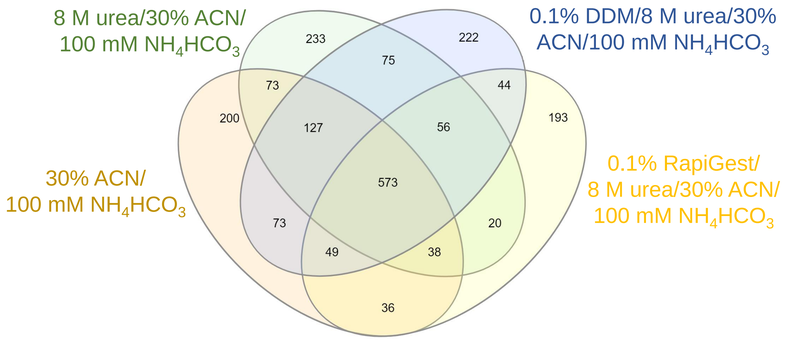

Figure 3. Overlap in protein identifications between the different extraction buffers for 15 µm thick GBM FFPE tissue.

Results

Effect of temperature increase on identified protein groups during urea incubation

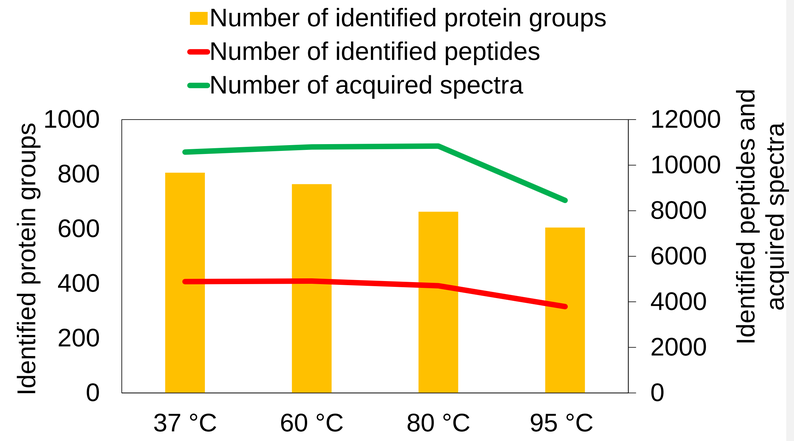

Figure 4. Protein identifications, identified peptides and acquired spectra for 15 µm thick GBM FFPE tissue at different temperatures using 8 M urea/30% ACN/100 mM NH_4HCO_3 as extraction buffer.

Biomarker candidates for GBM

- Nestin
- Gelsolin
- Cathepsin B
- Peripherin
- Glial fibrillary acidic protein
- Annexin A2
- Neural cell adhesin molecule 2
- Synaptic vesicle membrane protein vat-1 homolog

Conclusions

- Protein extraction from GBM FFPE tissue blocks was successful.
- Extraction buffer containing DDM yielded the highest number of identified proteins.
- Increasing of temperature during urea incubation resulted in a decrease in identified protein groups.

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