
Investigating the Impact of Pre-Analytical Variables on Protein Quality of Human Tissue Samples

March, 28-29, 2011

BRN Symposium „*Advancing Cancer Research
Through Biospecimen Science*“

Sibylle Gündisch

Institute of Pathology

Technische Universität München, Germany



Pre-Analytical Variables

Patient information
medication, anesthesia



Biopsy
Surgical resection

Fixation/Storage

Time

Times of

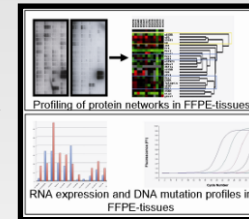
- vessel ligation
- specimen removal
- transport to pathology (temperature)

Fixation

- type of fixative
- fixation time

Storage

- duration
- temperature



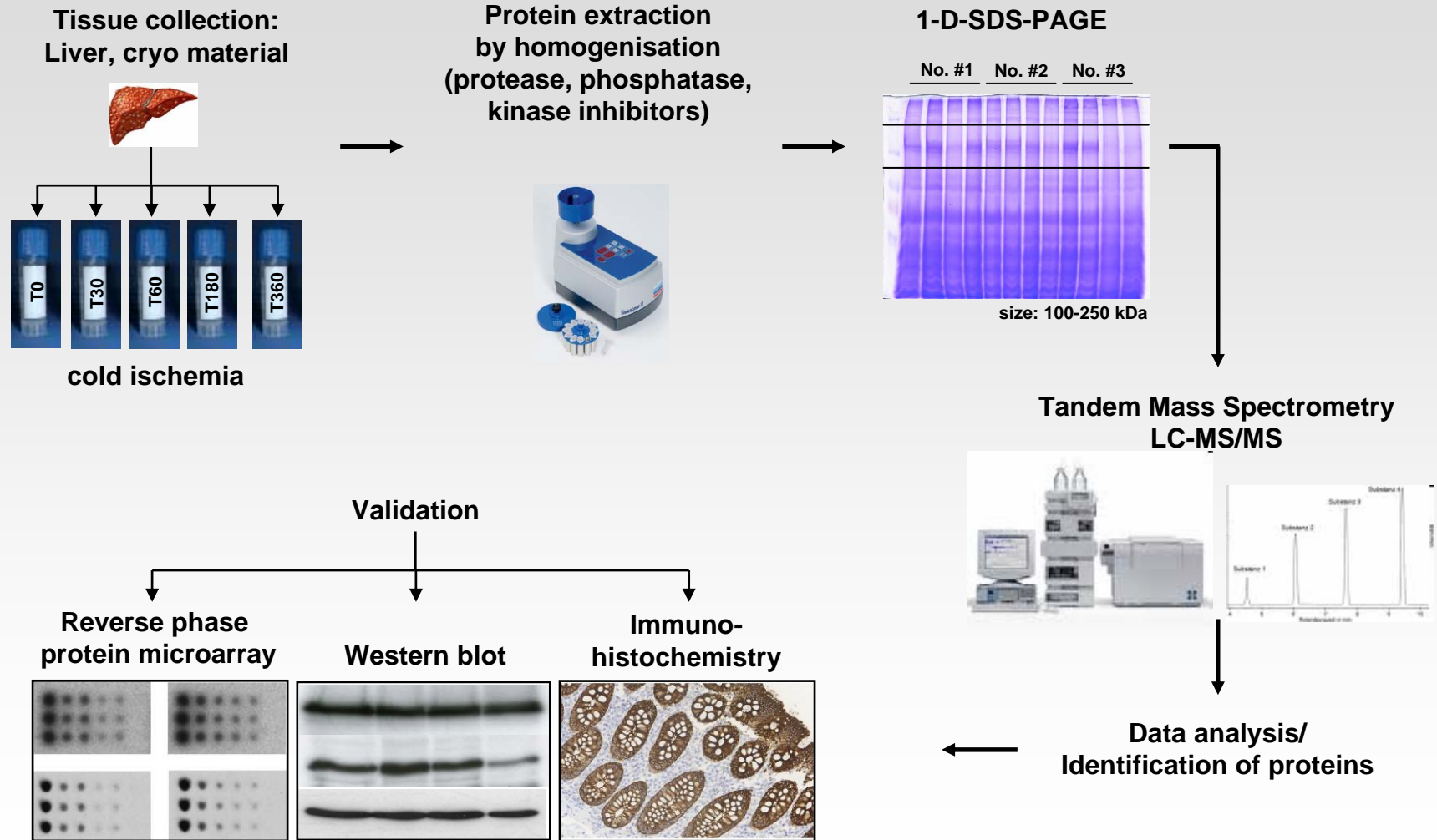
Molecular analysis

➔ Molecules might change during pre-analytical phase

➔ Basic prerequisite for biomedical research – High quality tissue specimen



Experimental setting



Results

- **Pilot study:**

- 1 patient sample (T0, T360)
- 290 identified proteins
 - 63 proteins significantly downregulated/degraded (21,7%)
 - 110 proteins significantly upregulated (37,9%)

➔ percentage of significantly differently expressed/regulated proteins in the extended study very small compared to the pilot study

➔ due to patient-to-patient variabilities

➔ **no global trend detectable towards up- or downregulation or degradation**

- **Extended study:**

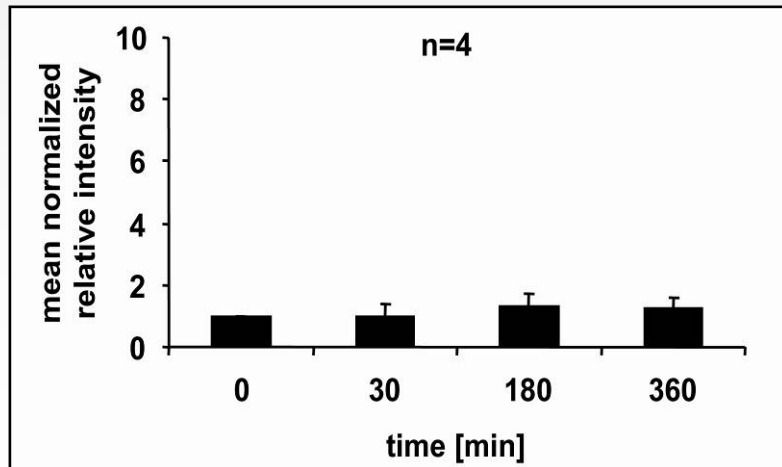
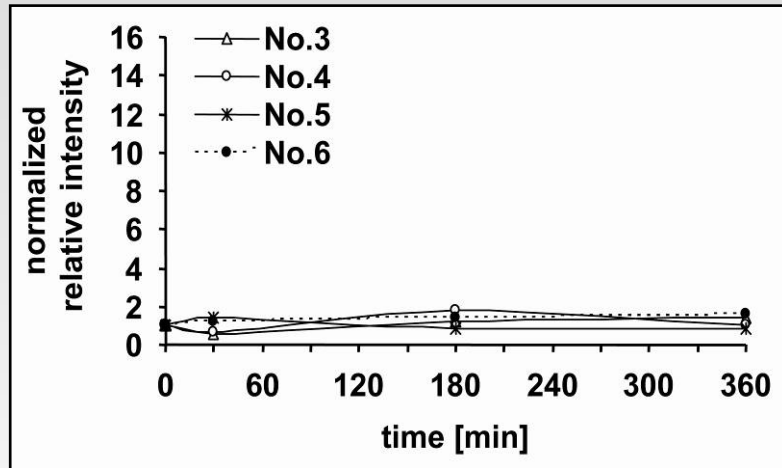
- 4 patient samples (biological replicates; T0, T30, T180, T360)
- 584 identified proteins
 - T0 → T360: 9 proteins significantly downregulated/degraded (1,5%)
 - T0 → T360: 21 proteins significantly upregulated (3,6%)

Results

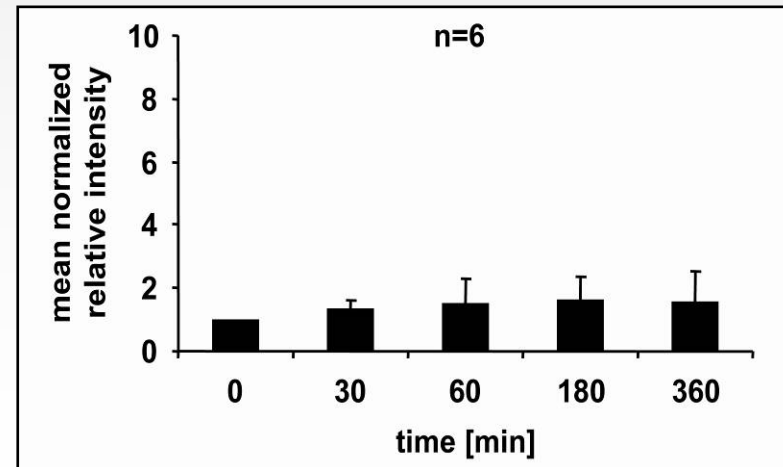
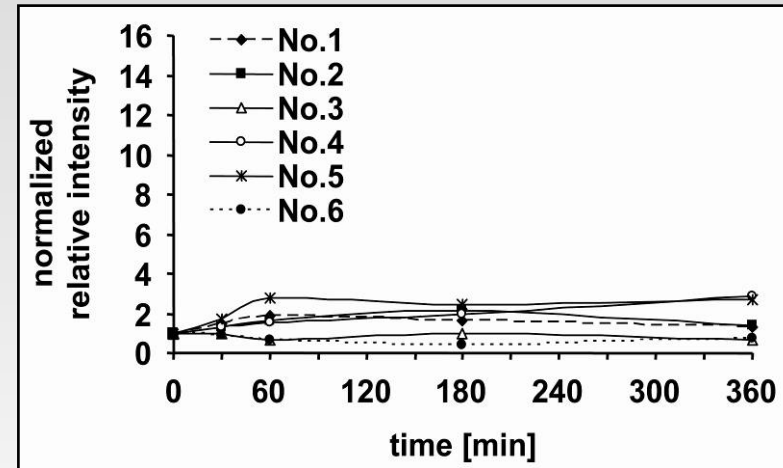
Mass Spectrometry

Validation by RPPA analysis

LC-MS/MS result of GAPDH:



RPPA result of GAPDH:



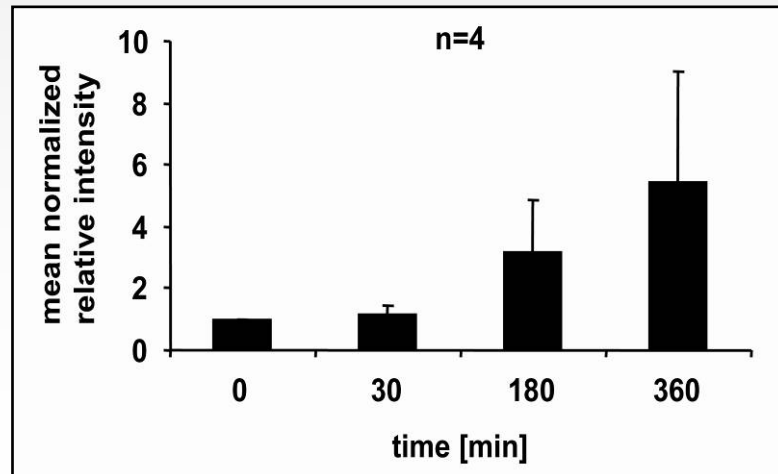
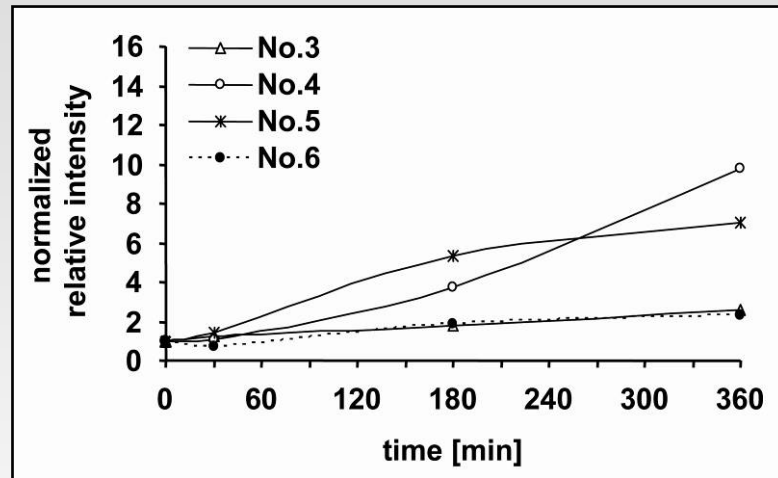
Samples were provided by the Institute of Pathology, Medical University of Graz, Austria

Results

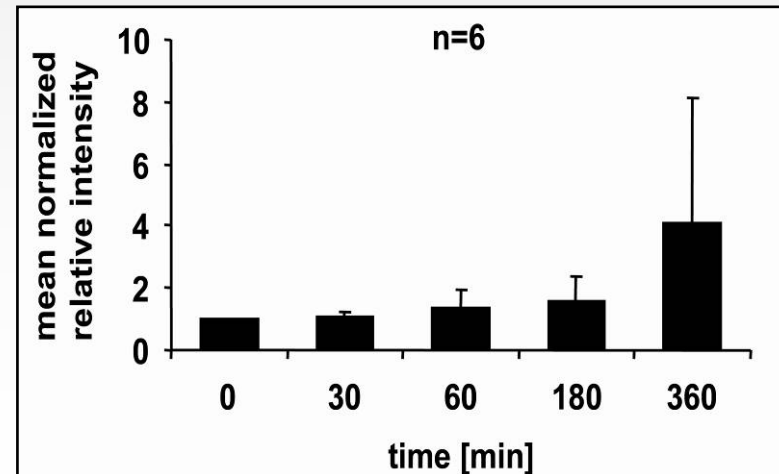
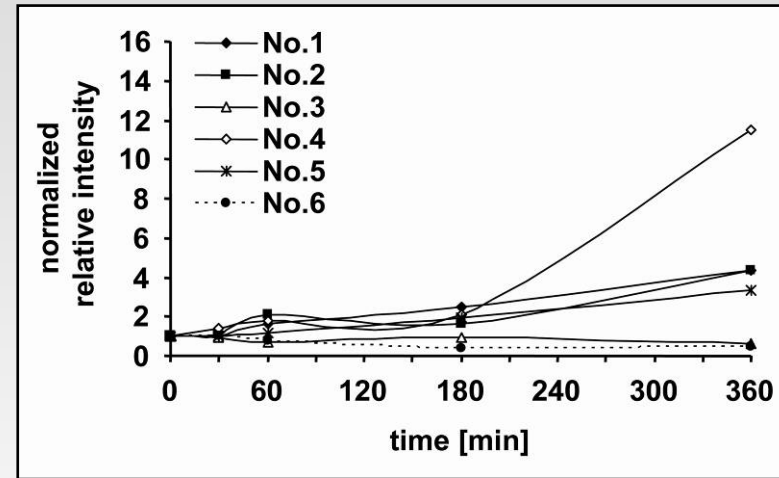
Mass Spectrometry

Validation by RPPA analysis

LC-MS/MS result of protein X:

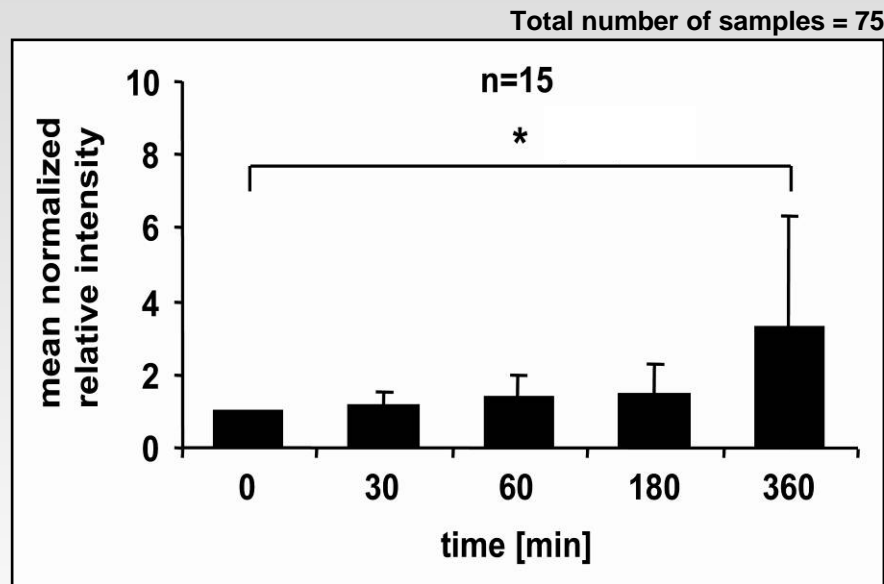


RPPA result of protein X:



Samples were provided by the Institute of Pathology, Medical University of Graz, Austria

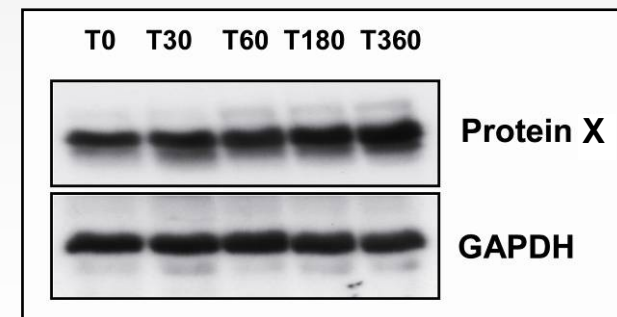
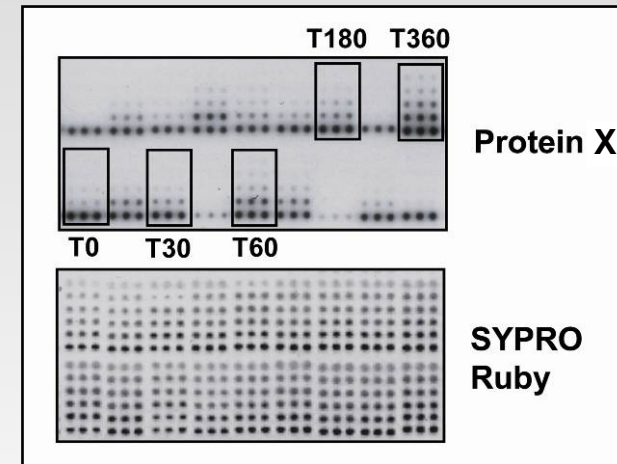
Validation by RPPA analysis in n=15 patient samples



➔ Protein X is significantly upregulated after 360 min

Representative RPPA and Western blot result

Patient sample No.2



Conclusions & Outlook

- **Proteome may be more stable than expected during first 60 min of ischemia**
- **Only a few proteins were found to be significantly up- or downregulated/degraded**
- **Data suggest that cold ischemia time up to 60 min has no major impact on tissue quality with regard to proteins**
- **Results have to be verified in different tissues (non-malignant and malignant)**
- **Comparison between different fixatives e.g. PAXgene Tissue System**

Thank you for your attention!

Acknowledgement



Pathology TUM:

Prof. Dr. H. Höfler
Prof. Dr. KF Becker

Dr. Katharina Malinowsky
Christa Schott
Claudia Wolff
Marina Grether

Dr. Enken Drecoll
Dr. Rupert Langer
Dr. Julia Slotta-Huspenina



Surgical department MRI:

PD Dr. Yves Harder
PD Dr. Robert Rosenberg



Core Facility Proteomics:

Dr. Stefanie Hauck
Dr. Hakan Sarioglu



www.spidia.eu

Dr. Uwe Oelmüller
Dr. Daniel Grölz
Prof. Dr. Kurt Zatloukal
Dr. Christian Viertler
Dr. Peter Riegman
Marcel Kap
Bas De Jong

