

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



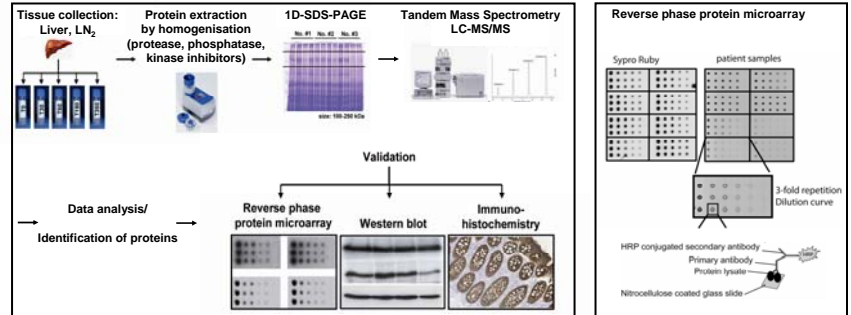
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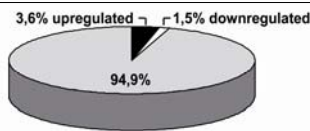
Background

The project SPIDIA (www.spidia.eu) supported by the European Union systematically addresses the impact of pre-analytical variables on the molecular portrait of clinical tissues. In this study we focussed on tissue quality under ischemic conditions with regard to protein expression. The overall goal of our project is to identify quality biomarkers for monitoring disease-unrelated changes in clinical tissue samples.

Material and Methods



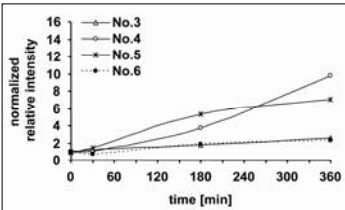
Results



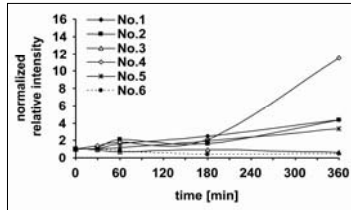
4 patient samples with 4 different ischemia time points (total number of samples n=16) were analyzed by tandem mass spectrometry

➔ No global trend detectable towards up- or downregulation or degradation

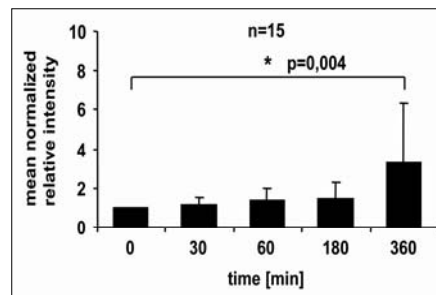
LC-MS/MS result of protein X:



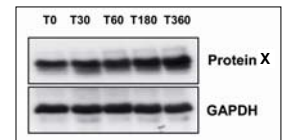
Validation by RPPA analysis:



Validation by RPPA analysis in n=15 patient samples (total number of samples n=75):

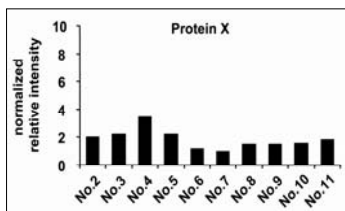
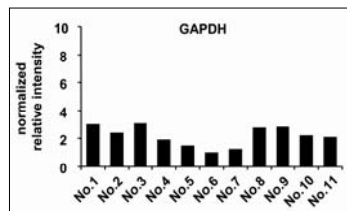


Representative Western blot result of patient sample No.2:



➔ Protein X is significantly upregulated after 360 min

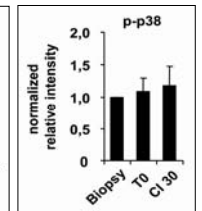
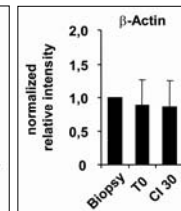
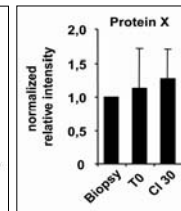
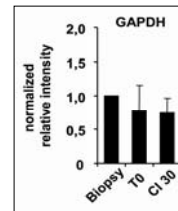
Investigation of patient-to-patient variabilities:



➔ Considerable patient-to-patient variabilities → > 3-fold differences

➔ Major hurdle in biomarker research

Only little differences in protein expression during warm ischemia:



➔ Variations between factor 0,5-1,5 were defined as no difference

➔ Little differences between intra-operative biopsy, warm and cold ischemia

Conclusion

- 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) and compared between different fixatives e.g. PAXgene Tissue System

Human tissue specimen were provided by the Institute of Pathology, Medical University of Graz, Austria and the Department of Pathology, Josephine Neufkens Institute, Erasmus Medical Center Rotterdam, The Netherlands with prior written informed consent by the patient. The work leading to this poster has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°222916

