Impact of Delayed Fixation on Protein Profiles in Clinical Tissue Samples
S. Gündisch\textsuperscript{1}, S. M. Hauck\textsuperscript{2}, H. Sarioglu\textsuperscript{2}, C. Viertler\textsuperscript{3}, M. Kap\textsuperscript{4}, C. Schott\textsuperscript{1}, P. Riegman\textsuperscript{4}, K. Zatloukal\textsuperscript{3}, K.-F. Becker\textsuperscript{1}

\textsuperscript{1}Institute of Pathology, Technische Universität München, Munich, Germany
\textsuperscript{2}Department of Protein Science, Helmholtz Zentrum München, Neuherberg, Germany
\textsuperscript{3}Institute of Pathology, Medical University of Graz, Graz, Austria
\textsuperscript{4}Department of Pathology, Josephine Nefkens Institute, Rotterdam, The Netherlands

Background: The European consortium SPIDIA (www.spidia.eu) systematically addresses the impact of pre-analytical factors on the molecular portrait of clinical tissue samples. In this study we focussed on potential changes of the proteome under ischemic conditions.

Methods: Homogeneous human liver samples were collected in the operating theatre under different ischemic conditions and cryopreserved. Biological replicates were analyzed using one-dimensional gel electrophoresis followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Validation studies were performed by reverse phase protein array (RPPA) and Western blot.

Results: The LC-MS/MS results indicate that only a small percentage of proteins was significantly differentially regulated among the different ischemia time points. Interestingly, we found that Cytokeratin 18 was upregulated after 360 minutes of ischemia, while in contrast GAPDH expression was found to be very stable. Analysis by RPPA and Western blot confirmed these data. Importantly, we found a high patient-to-patient variability with regard to protein expression.

Conclusion: Since we could not detect a consistent global trend towards up- or downregulation of the proteome upon ischemia, we conclude that the proteome seems to be more stable than expected in clinical tissue samples. However, the observed high patient-to-patient variability might be a major hurdle in future protein biomarker discovery programs.