Talk Abstract

Deutsche Pathologentagung 2012
Berlin, Germany, May 31- June 03, 2012

Delay to preservation does not induce a systematic phosphoprotein response during tissue processing

S. Gündisch[1,4,5], C. Schott[1,4,5], K. Grundner-Culemann[2,5], M. Machatti[2,5], D. Groelz[3,4], C. Schaab[2,5], A. Tebbe[2,5], KF Becker[1,4,5]
[1]Institute of Pathology, Technische Universität München, Munich, Germany
[2]Evotec AG, Martinsried, Germany
[3]PreAnalytiX GmbH, Hombrechtikon, Switzerland
[4]The SPIDIA Consortium, www.spidia.eu
[5]Munich Biotech Cluster m4, www.m4.de

Aims: The quality of tissue samples can have a significant impact on analytical data sets for biomarker research. In particular, posttranslational modifications such as phosphorylation need to be systematically investigated in that phosphorylated protein levels indicate the activation status of signal transduction pathways controlled by kinases. However, little is known about the impact of pre-analytical factors on phosphoprotein stability. The aim of this study was to characterize the potential effects of delayed preservation and different preservation methods on the stability of phosphoproteins using targeted and non-targeted proteomic approaches.
Methods: Murine and rat liver samples were exposed to different ischemic conditions before preservation and either cryopreserved, formalin-fixed or fixed with the PAXgene Tissue System, a new non-crosslinking formalin-free fixative. The phosphoproteome was analyzed using quantitative tandem mass spectrometry (LC-MS/MS) and reverse phase protein array (RPPA) technology.
Results: The phosphoproteomic analysis of ischemic mouse liver tissue samples by LC-MS/MS indicated no significant global alterations of more than 5000 phosphosite-ratios analysed during 60 minutes of delayed cryopreservation. The analysis of ischemic rat liver tissue samples by RPPA revealed similar results as investigated phosphoproteins, including phospho-Akt, phospho-p38 MAPK or phospho-p44/42 MAPK, showed very stable profiles during the time-course experiment, independent of the preservation method applied.
Conclusion: Since we could not detect significant global changes of the phosphoprotein profiles, neither with a targeted nor with a non-targeted approach, we conclude that the phosphoproteome seems to be more stable than expected with regard to delayed preservation. This allows accurate quantitative measurements of the activation state of signalling pathways of tissue samples which had not been immediately preserved. This result is essential for the development of new targeted therapies involving kinase inhibitors which have recently been a focus in the field of personalized medicine. Studies are ongoing

to validate our results in human tissue samples as inter-patient variability may occur which is absent in our well controlled model systems.