Poster Abstract

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Impact of pre-analytical factors on protein and phospho-protein profiles in tissue samples

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Background: Precise quantitation of protein biomarkers in tissues has great potential for the development of personalized molecular targeted therapies. However, little is known about the impact of pre-analytical factors on protein stability. One aim of the Munich Biotech Cluster M[4] is to develop standard operating procedures for asservation of tissue specimen. In this study we focussed on potential changes of phospho-proteins with regard to delayed fixation.
Methods: Murine and rat liver samples were collected under different ischemic conditions and either cryopreserved, formalin-fixed or fixed with the PAXgene Tissue System, a new formalin-free fixative, that is being evaluated by the European FP7 consortium SPIDIA (www.spidia.eu). The phosphoproteome of biological triplicates was analyzed using quantitative mass spectrometry (LC-MS/MS) and reverse phase protein array (RPPA) technology.
Results: The phosphoproteomics analysis of ischemic mouse liver tissue samples indicated no significant global alterations of more than 5000 phosphosite-ratios analysed during 60 minutes of delayed cryopreservation. The analysis of differently fixed ischemic rat liver tissue specimens by RPPA revealed similar results as phosphoproteins, including p-Akt, p-p38 MAPK or p-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 phospho-protein profiles, neither with a targeted nor with a non-targeted approach, we conclude that the phosphoproteome seems to be more stable than expected in ischemic tissue samples. This allows accurate quantitative measurements of the activation state of signalling pathways which is essential for the development of targeted therapies involving kinase inhibitors. However, our results need to be validated in human tissue samples as inter-patient variability may occur which is absent in our well controlled model systems.