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

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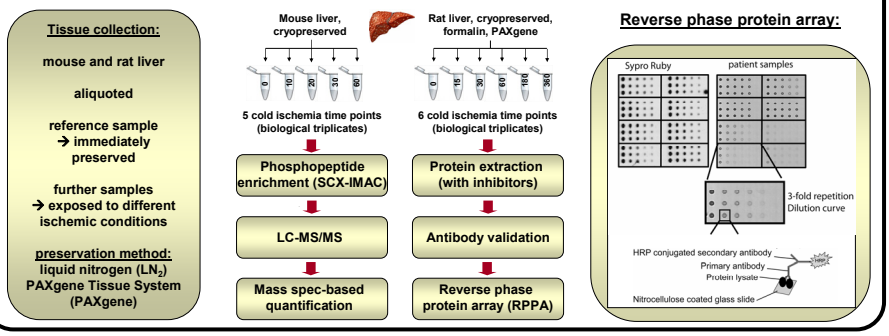
Background

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.**

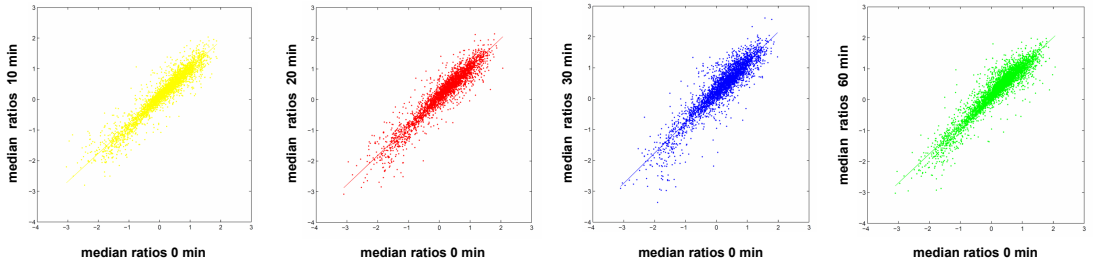
Material and Methods



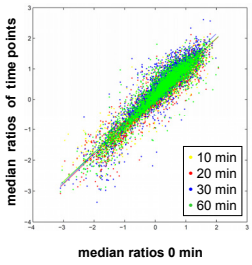
Results

Quantitative tandem mass spectrometry (mouse data):

Correlation of phosphosite-ratios of ischemic (10, 20, 30, 60 min) mouse liver samples against the reference sample (0 min):

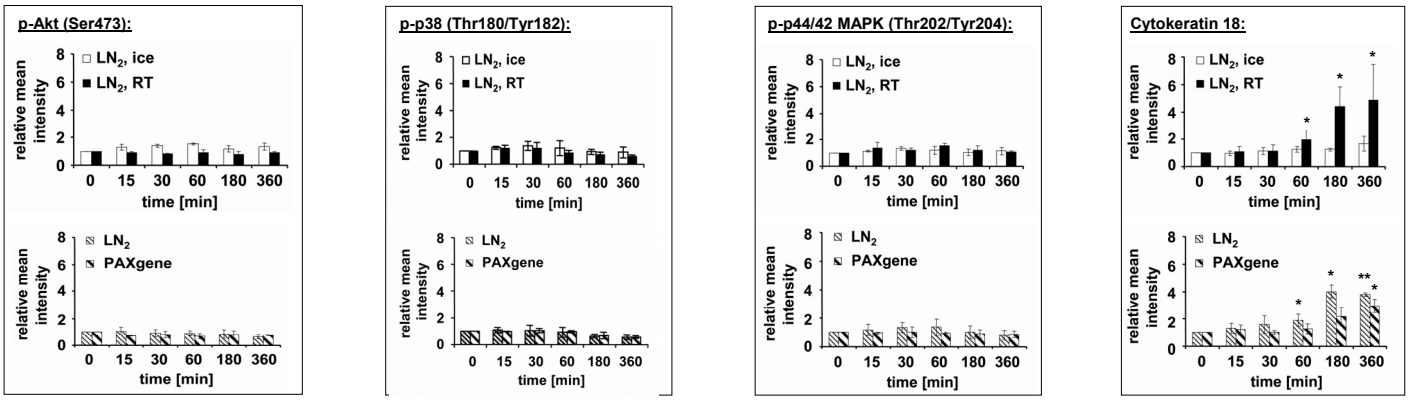


Overlay of all time points:



➡ **no significant global alterations of more than 5000 phosphosite-ratios analysed during 60 minutes of delayed cryopreservation**

Reverse phase protein array (rat data):



➡ **phosphoproteins, including phospho-Akt, phospho-p38 MAPK or phospho-p44/42 MAPK, showed very stable protein profiles during the whole time-course experiment, independent of the preservation method applied**

➡ **in contrast to Cytokeratin 18 which shows an increase in signal intensity upon delayed preservation which could be prevented by cooling of the tissue samples on ice during the time-course experiment**

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 signaling 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.

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