Evaluation of PAXgene-fixed, Paraffin-embedded Tissues for Proteomic Applications
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Aims: For molecular diagnostics and personalized medicine, protein biomarkers need to be precisely measured in clinical tissue samples. In formalin-fixed and paraffin embedded (FFPE) tissues, protein analysis is still challenging. In this study we evaluated a novel tissue fixation system for better integration of morphological and molecular analysis, focussing on protein assays.

Methods: Different murine and human tissue samples were fixed with a novel formalin-free tissue fixative, PAXgene tissue fixation and stabilization reagents. Proteins were analyzed by Coomassie staining, Western blotting, reverse phase protein microarrays (RPPA) and matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS). Morphology and immunohistochemistry were evaluated and RNA quality was assessed by PCR amplification assays.

Results: We were successful in extraction of non-degraded and immunoreactive proteins from PAXgene-fixed tissue specimens. We analyzed for example E-cadherin, Hsp70 and beta-actin and phosphorylated proteins, including p-Akt, p-Erk-1/2, and p-NFkB. Recovered proteins showed very similar properties when compared to cryopreserved samples by Western blotting and RPPA and were superior to proteins from FFPE samples. Furthermore, the spectra of MALDI-IMS analysis were similar to cryopreserved samples which were visualized by insulin and glucagon expression in pancreatic tissue. Finally, morphology was comparable to FFPE samples whereas RNA was far better preserved in PAXgene-fixed samples.

Conclusion: PAXgene tissue fixation and stabilization reagents have great potential to serve as a novel multimodal fixative for modern pathology, enabling extensive protein biomarker studies on clinical tissue samples.