Poster Abstract

BRN symposium
Bethesda, Maryland, USA, February 22-23, 2012

The impact of tissue pre-analytics and a new stabilisation technology on the quality of tissue-based molecular studies

C. Viertler[1], D. Groelz[2], K. Kashofer[1], S. Gündisch[3], P. Riegman[4], R. Winther[5], R. Wyrich[6], M. Kruhoffer[7], KF Becker[3], U. Oelmüller[6], K Zatloukal[1]
[1] Institute of Pathology, Medical University of Graz, Austria
[2] PreAnalytiX GmbH, Hombrechtikon, Switzerland, c/o QIAGEN GmbH, Hilden, Germany
[3] Institute of Pathology, Technical University Munich, Germany
[4] Department of Pathology, Josephine Nefkens Institute, Rotterdam, The Netherlands
[5] Dako Denmark A/S, Glostrup, Denmark
[6] QIAGEN GmbH, Hilden, Germany
[7] AROS Applied Biotechnology A/S, Aarhus, Denmark

Background: Molecular characterization of human disease requires analysis of multiple parameters ranging from classical histopathological features to a broad spectrum of molecular biomarkers. The morphological characterization is routinely based on the analysis of formaldehyde-fixed and paraffin-embedded (FFPE) tissues but it is known that formalin fixation impairs molecular analyses which typically require frozen tissue samples. Within the EU FP7 project SPIDIA we evaluated a new technology for combined tissue diagnostics and the impact of several pre-analytical variables on tissue sample quality and subsequent molecular analyses.
Methods: FFPE samples were compared to alternative fixatives, including a novel technology for simultaneous preservation of morphology and biomolecules (PAXgene Tissue System), and corresponding snap-frozen tissue samples served as reference. Morphology, antigenicity and different biomolecules were investigated with a focus on nucleic acids preservation.
Results: Established methods for quality control of RNA (e.g. 28s:18s ratio, RIN value) were well-suited for frozen tissue, but a more detailed analysis, like a qPCR assay based on different amplicon length, was needed to estimate the suitability of RNA from paraffin-embedded tissues for downstream applications. Results of qPCR were sensitive to RNA degradation introduced by pre-analytical procedures such as time or type of fixation and storage. In contrast to FFPE, PAXgene-fixed and paraffin-embedded (PFPE) samples showed outstanding RNA preservation and strong correlation of multiple mRNA and microRNA profiles with snap-frozen samples as revealed by qPCR and microarray analysis. DNA isolated from PFPE tissues was of high molecular mass and well-suited for long-range and multiplex PCR, and different sequencing techniques. Proteins extractions from PFPE samples showed comparable yield and preservation of phosphorylation levels to cryo-preserved samples.
Conclusion: The quality of tissue-based molecular studies can only be defined in the context of the pre-analytical procedures. The excellent preservation of biomolecules and morphology in PFPE samples provides new opportunities for comprehensive tissue diagnostics, biomarker discovery and reliable molecular analyses whenever a collection of snap-frozen material is impossible.