Talk Abstract

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Pre-analytical Parameters Impacting on Tissue-based Biomarkers

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Background: Molecular characterization of human cancer requires analysis of multiple parameters ranging from classical histopathological features to a broad spectrum of molecular biomarkers. The morphological characterization of tumors is based on the analysis of formaldehyde-fixed and paraffin-embedded (FFPE) tissues, whereas analysis of molecular biomarkers typically requires frozen tissue samples, the quality of which is affected by a variety of pre-analytical parameters. Furthermore, in the context personalized medicine there is increasing need for combined morphological and molecular diagnostics from the same tissue sample, especially when collection of freshly frozen material is impossible for medical, ethical or logistic reasons.
Methods: To assess pre-analytical variables related to tissue quality such as ischemia, sample processing, fixation method, fixation time, embedding and storage, tissue specimens were divided in several aliquots and processed in parallel according to different protocols. FFPE samples were compared with PAXgene-fixed and paraffin-embedded (PFPE) samples. PAXgene is a novel fixative that should simultaneously preserve morphology antigenicity and biomolecules. Snap-frozen tissue served as reference. Comparative studies of morphology, antigenicity and nucleic acids preservation were performed with a focus on RNA quality using electropherograms, spectroscopy, a qRT-PCR assay based on different amplicon lengths, and gene signature arrays for 92 cancer-related genes.
Results: Results demonstrated that established methods for quality control of RNA, such as the ratio of the ribosomal 28s:18s RNA or the RIN value are good parameters for frozen tissue but do not readily correlate with PCR amplification efficacy of RNA isolated from paraffin-embedded tissues. Quantitative assessment of RT-PCR efficacy for different amplicon lengths was better suited for the assessment of RNA quality isolated from paraffin-embedded tissues. FFPE tissues showed ct values differing from 4 up to 15 cycles (depending on the amplicon length) from the corresponding ct value for snap-frozen samples, reflecting RNA fragmentation and modification. Furthermore, the 92 cancer-pathway associated gene signature arrays (TaqMan) revealed major gene to gene variations (up to 7 cycles) between FFPE and fresh frozen tissues that could be attributed to RNA modification during formaldehyde fixation whereas the downstream pre-analytical processes has only minor effects. In contrast to FFPE, PFPE tissues constantly demonstrated excellent RNA quality, similar to RNA

from fresh frozen tissues, and at the same time resulted in good preservation of morphology and antigenicity.
Conclusion: Pre-analytical parameters have a major impact on molecular tissue diagnostics and assay results. Consequently the quality of a tissue-based biomarker can only defined in the context of the pre-analytical procedures. The excellent preservation of biomolecules in PFPE samples besides well-preserved morphology and antigenicity provides the opportunity to analyze a broad spectrum of biomarkers and classical histopathological diagnosis from the same tissue sample. This markedly facilitates biomarker research and the future application of molecular biomarkers in a routine clinical context.