A New Technology for Simultaneous Preservation of Biomolecules and Morphology in Tissues Facilitates Biomarker Development

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Background

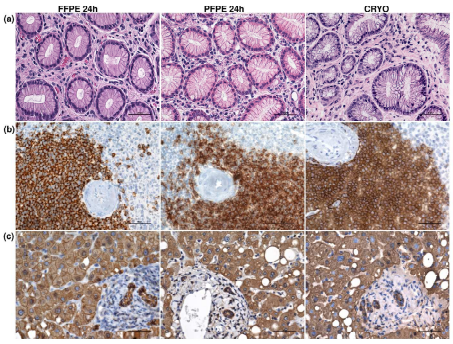
Molecular characterization of human disease requires analysis of multiple parameters ranging from histopathology to a broad spectrum of molecular biomarkers. The morphological characterization is based on the analysis of formaldehyde-fixed and paraffin-embedded (FFPE) tissues but it is known that formalin fixation impairs molecular analyses which typically require frozen tissue samples. In the context of personalized medicine, upcoming molecular diagnostics and omics-technologies, there is an increasing need for combined morphological and molecular analyses from the same tissue sample. Within the European FP7 project SPIDIA we developed and tested a new technology (PAXgene Tissue) for high-quality preservation of biomolecules and morphology in paraffin-embedded tissue samples.

Methods

Corresponding samples from different human (non-) malignant tissues were either fixed in buffered formaldehyde or with PAXgene Tissue and paraffin-embedded (PFPE), snap-frozen tissue samples served as reference. In a comparative study we investigated the preservation of morphology, antigenicity, nucleic acids and (phospho-) proteins.

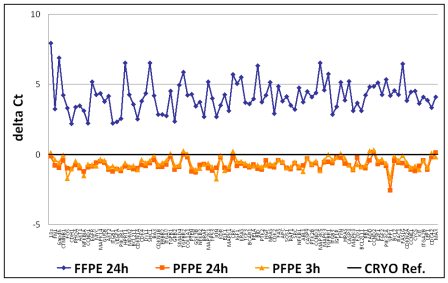
Results

Morphology and Antigenicity



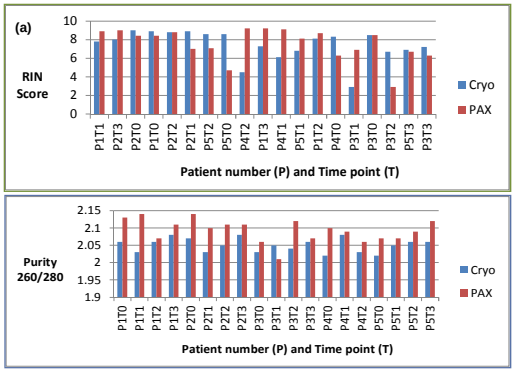
PFPE samples show well-preserved morphology and antigenicity, comparable to FFPE samples. For some antibodies less harsh retrieval procedures can be used.
(a) H&E staining of human stomach tissue. (b) CD 20 staining of human spleen tissue (c) K8/18 staining of human liver.

Nucleic Acids



High correlation of gene signature in PFPE and cryo-preserved samples (R^2=0,99), **but decreased correlation** (R^2=0,89) **and major gene-to-gene variations in FFPE samples.**
Gene expression analysis of corresponding human liver samples by qRT-PCR on predefined TaqMan array "Human Molecular Mechanisms of Cancer" plate. Delta Cts were calculated using the frozen sample as reference.

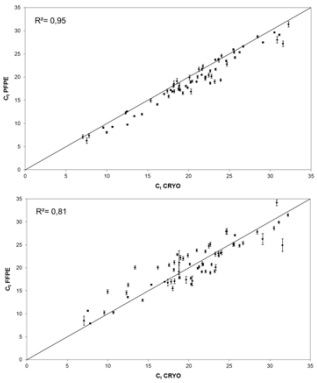
Impact of ischemia time and stabilization method analysed with Affymetrix Human Genome U219 Array on the GeneTitan hybridization, wash and scanning station. Liver needle biopsies from 5 patients (P1-5), exposed to different ischemia time points (T0-3), were either snap-frozen (Cryo) or fixed and stabilised with PAXgene Tissue (PAX). (a) RNA quality control (b) GDM analysis.



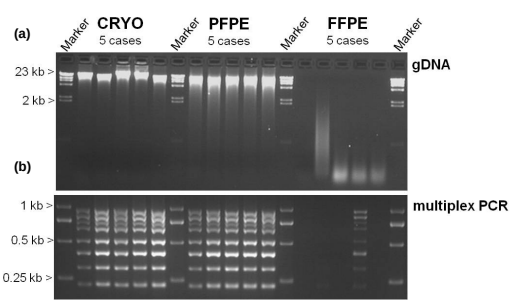
Quality control using Agilent Bioanalyzer shows comparable RNA integrity (RIN score) and purity (OD 260/280 ratio) of PFPE and cryopreserved samples.

(b)

High-throughput expression profiling reveals high correlation between PAXgene-fixed and snap frozen samples.
GDM analysis showing the Euclidian distance between samples based on expression values from all genes. The variable that contributes most to the distance between samples is the individual and not ischemia time or sample stabilization method.

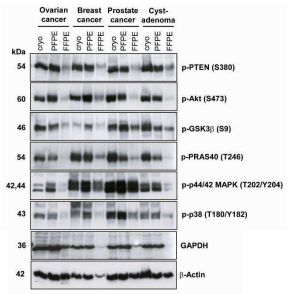


High correlation of microRNA expression in PFPE and snap-frozen samples (R^2= 0.95), **whereas FFPE samples showed a lower correlation** (R^2= 0.81). MicroRNAs from corresponding aliquots of three colon cancer cases were quantified by real-time RT-PCR on a TaqMan 7700.

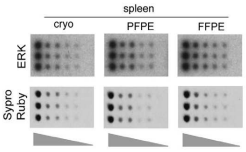


DNA integrity& performance in multiplex PCR of PFPE samples.
(a) High molecular mass bands indicating good DNA integrity were visible for PFPE and cryo-preserved samples but not for FFPE samples. Genomic DNA extracted from corresponding FFPE, PFPE, and snap-frozen (CRYO) samples of 5 human breast cancer cases was separated on 1% agarose gels and visualized with ethidium bromide.
(b) Multiplex PCR of eight fragments of different human genes. PRNP (222 bp), CD79b (310 bp), cKIT (414 bp), AGTR2 (523 bp), CD14 (662bp), CD40 (756 bp), CD59 (845 bp) and CD19 (955 bp).

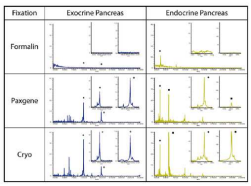
Proteomic Applications



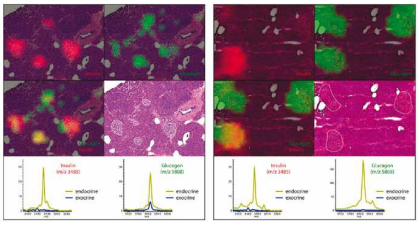
Western blot analysis of phosphoproteins in PFPE human tissue samples: **visible protein bands of PFPE extracts are comparable to snap frozen samples and the phosphorylation levels are well-preserved.**



PFPE samples are comparable to cryo-preserved samples in reverse-phase protein microarray (RPPA) experiments.



MALDI imaging mass spectrometry.
In contrast to FFPE, PFPE and cryopreserved pancreatic samples display a multitude of peaks (upper panel). Visualization of Insulin and Glucagon expression (lower panel: left/PFPE, right/cryopreserved samples).



Conclusions

New Tissue Stabilisation Technology PAXgene Tissue provides

- Preservation of **morphology and antigenicity** resembling FFPE samples.
- Excellent **RNA** quality and strong correlation of multiple mRNA profiles with snap frozen samples in qPCR and microarray analysis.
- High molecular mass **DNA**, well-suited for long-range and multiplex PCR, and different sequencing techniques.
- Comparable preservation of **(phospho-) proteins** with snap- frozen samples.

Innovative Aspects

- Simultaneous high-quality preservation of biomolecules and morphology in clinical tissue samples.
- Direct correlation of morphological disease phenotypes with alterations of biomolecules.
- Multimodal biomarker studies in a routine clinical setting.
- Molecular analyses of lesions where a collection of snap-frozen material is impossible.

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References: Viertler et al. J Mol Diagn. 2012 Sep;14(5):458-66. Epub 2012 Jun 28. Ergin et al. J Proteome Res. 2010 Oct 1; 9(10):5188-5196.

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