Background

Current methods for tissue preservation involve fixation with formalin or snap-freezing in liquid nitrogen. Formalin preserves tissue by cross-linking proteins and nucleic acids, but this cross-linking cause irreversible chemical modification and damage to proteins and nucleic acids, particularly RNA. While preservation of tissue morphology allows for standard histopathological analysis, it is usually accompanied, restriction of unfolding, of frozen samples is technically demanding, and specific equipment is required.

PAXgene Tissue (PAXgene) is a new, formalin-free fixation technology. PAXgene fixed, paraffin-embedded (PFPE) tissue is suitable for conventional immunohistochemical and immunofluorescent staining as well as for extraction of high quality nucleic acids and proteins including phosphoproteins.

Material & Methods

Cases of human cancers were divided and fixed either for 24 hours in neutral buffered formalin, or in a solution with a ratio of 3:1 formalin and LN2 (Formalin+LN2, a mixture of formalin and LN2). Nucleic acids were isolated and analyzed in RT-qPCR, by agarose gel electrophoresis, long-range, and multiplex PCR (Tab. 1). Proteins and phosphoproteins were extracted by western blot analysis. 17β-Hydroxyprogesterone (β-Hydroxyprogesterone), β-ACTIN (Sigma-Aldrich), GAPDH (Stressgen Biomol), Phospho-PRAS40, and Phospho-KAP1 (Cell Signaling Technology).

Results

Morphology of PFPE samples and IHC staining intensities were similar to that for FFPE tissue (Fig. 1). High correlation of RNA and miRNA expression between PFPE and FFPE and between FFPE and LN2 was observed (Fig. 2). Figure 3 shows an example of electrophoretic profiles of RNA and miRNA obtained from mirrored FFPE, PFPE and LN2 snap-frozen specimens of breast cancer. Figure 4 shows dot blot analysis using specific DNA markers for preservation of the phosphoproteome in PFPE human tissue samples. Phosphoproteins can be isolated from PFPE tissue.

Conclusion

Data presented in this poster demonstrates that PAXgene fixed, paraffin-embedded PFPE tissue is suitable for routine and research purposes. Furthermore, phosphoproteins can be isolated from PFPE tissue.

References


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