P18 Proteomic analysis of formalin fixed tissues for the investigation of prostate cancer

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Introduction & Objectives: As proteins represent cell function, proteomic analysis of prostate tissues may elucidate the mechanisms of prostate cancer (PCa) progression. To that end, formalin-fixed paraffin-embedded (FFPE) tissues are valuable resources, but associated with large analytical difficulties due to protein cross-linking. Our aim is to characterize tissue protein changes associated with castration resistant PCa. This study focused on the development of a reliable protocol for the proteomic analysis of FFPE tissues which was followed by a pilot study using FFPE PCa clinical samples to investigate whether the optimized protocol can provide biologically relevant data in the context of PCa.

Materials & Methods: Archival FFPE mouse tissues were processed using seven protein extraction protocols including combinations of homogenization means (beads, sonication, boiling) and buffers (Tris-HCl and Urea-Thiourea). The proteomics output was evaluated by SDS electrophoresis and high resolution LC-MS/MS analysis.
FFPE tissues (3 sections, 15μm each per sample) from 10 patients with PCa corresponding to tumor (GS=6 or GS≥8) and adjacent benign regions were processed with the optimized protocol. Extracted proteins were analyzed by GeLC-MS/MS followed by statistical and bioinformatics analysis using Cytsoscape and Open Targets tool.

Results: A combination of cell lysis by Tris-HCl, SDS and DTE with bead homogenization, sonication and boiling provided most efficient protein extraction from FFPE tissues based on protein identifications and reproducibility. Comparison between the FFPE and matched FF tissues showed a substantial overlap in protein identifications (1106 common out of FF:1214 IDs and FFPE:1249 IDs) with a strong correlation in relative abundances (r_S=0.846, p<.001). Proteins significantly deregulated between PCa GS≥8 and PCa GS=6 represented extracellular matrix organisation, phosphorylation and gluconeogenesis pathways, while proteins deregulated between cancerous tissues and benign counterparts, reflected increased translation, peptide synthesis and protein metabolism in the former, as expected. Further disease association analysis using the Open Targets platform showed that 60% of the differentially expressed proteins in cancer versus normal adjacent were significantly associated with PCa.

Conclusions: These results support the relevance of the proteomic findings in the context of PCa and the reliability of the optimized protocol for proteomics analysis of FFPE material. A large scale study including 160 clinical FFPE prostate cancer and benign tissues to define protein changes associated with aggressive PCa, is planned.