Development of an Automated Exosome Isolation Procedure for Determining Prostate Cancer Aggressiveness

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Almost 230,000 men are diagnosed with prostate cancer annually. However, over 900,000 men will undergo biopsy as part of routine screening to assess the presence of cancer. The majority of these cases are of low risk disease which is unlikely to cause significant morbidity and mortality. Thus, a biomarker to distinguish indolent from aggressive disease could potentially decrease the complications and morbidity related to prostate biopsies and ambiguity surrounding definitive treatment.

Exosomes are small (30-200 nm) membrane-bound vesicles secreted by all cell types. They are found in many clinical samples, including serum/plasma, urine, cerebrospinal and other fluids such as saliva, tears, and breast milk. They harbor a multitude of biomarkers and their contents reflect both cell of origin and disease status. As such, they can serve as a "liquid biopsy" to assess disease/health status in real-time. In addition, they can provide insights into disease mechanisms and characteristics (e.g. including prognostic information) for prostate cancer.

We have developed and optimized a procedure for the isolation and analysis of exosomes derived from serum. Our procedure entails chemical precipitation and affinity chromatography using antibodies to specific membrane proteins. We have utilized this procedure to compare exosome protein profiles derived from patients with metastatic disease and those who have undergone radical prostatectomy. Western blot analysis using α-CD63 antibody (CD63 is an exosome surface protein) demonstrates that our preparation contains exosomes, identical to those obtained using the standard procedure, ultracentrifugation, which is more cumbersome, lengthy, and non-specific. Size fractionation shows that mean size of particles= 80-150 nm. Mass spectrometric based proteomics analysis via 1D LC-MS/MS and multidimensional protein identification technology (MudPIT) was performed after trypsin digest. Sequenced peptides were mapped to the human proteome. We identified >800 proteins from this sample set. A

subset of proteins comprises a 44-protein signature that can distinguish these two patient groups. Using GeneOntology (GO) analysis, the 44 proteins can be classified into eight major functions, such as cytoskeleton maintenance and signal transduction. Included in this list are several proteins already shown to be involved in prostate cancer progression, invasion, and metastasis (actin filament associated proteins, calcium binding proteins e.g. S-100, etc.), as well as newly identified biomarkers (e.g. synaptopodin-2).