Prostate Cancer Foundation Retreat 2025 Poster Abstract

Unlocking the potential of large oncosomes as a liquid biopsy tool for prostate cancer through next generation capture and detection methods

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Background:

Extracellular vesicles (EVs) are membranous structures released from cells through endocytic pathways or plasma membrane shedding. They are heterogenous in size, functions and molecular composition, contributing to diverse physiological and disease processes, including cancer progression. Large oncosomes (LOs) are an atypically large subtype of EVs shed by aggressive cancer cells and enriched in tumor-derived cargo. While LOs are elevated in the circulation of patients with metastatic prostate cancer, highlighting potential diagnostic and prognostic value, the efficient and specific recovery of LOs from biofluids remains challenging. Traditional isolation methods, including size exclusion chromatography (SEC) and density gradient ultracentrifugation (DGUC), have been optimized for small EVs (S-EV) but their utility for recovering large EV (L-EV) subtypes is unclear. This study characterized S-EV and L-EV subpopulations from cancer cells and patient plasma, comparing the efficiency of traditional approaches and next-generation immunoaffinity capture strategies.

Methods:

Three EV subsets were pelleted from PC3 conditioned media by differential ultracentrifugation (dUC: 2.8K, 10K, 100K *g*) followed by SEC using Izon qEV70 columns or Optiprep DGUC. Particle concentration and size were quantified by microfluidic (MRPS) and tunable (TRPS) resistive pulse sensing and western blotting detected markers of S-EVs and L-EVs. Annexin V-coated magnetic beads were used to capture L-EVs and analyzed for LO-enriched surface markers by flow cytometry. Surface markers were also imaged by dSTORM super-resolution microscopy and targeted for specific capture on the HB^{EV}-Chip. Marker expression was also explored by western blotting of dUC fractions from patient plasma and healthy donors.

Results:

EV pellets obtained at increasing centrifugation speeds differed in abundance, size, morphology, and marker expression. L-EVs eluted in lower yields than S-EVs and overlapped across at least two SEC fractions, suggesting SEC is biased towards S-EVs and poorly resolves larger subpopulations. Both DGUC and SEC resulted in substantial particle loss. Annexin V-coated beads captured 99% of L-EV and improved detection of vesicles positive for LO markers VDAC1, RPN2 and STEAP1. dSTORM confirmed the colocalization of VDAC1 and RPN2 on L-EVs. The HB^{EV}-Chip functionalized with antibodies to L-EV (VDAC1, RPN2) or S-EV (CD81, ICAM) proteins successfully captured vesicles from the 10K and 100K fractions, respectively, with 10K isolates enriched 8-fold in LO-associated mitochondrial transcripts. dUC fractionation of plasma samples verified the distribution of markers across EV subpopulations from patients and healthy donors.

Conclusions:

This study underscores the limitations of conventional EV isolation and the need for innovative, scalable strategies to recover specific subpopulations relevant for clinical translation. Immunoaffinity-based methods efficiently captured rare, tumor-derived EVs, such as LO, from heterogenous mixtures, improving detection of prostate cancer biomarkers. Through rigorous characterization and orthogonal approaches, these methods hold promise for sensitive, non-invasive monitoring of tumor biology to facilitate early diagnosis and monitoring cancer progression.

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Conflicts of Interest Disclosure Statement:

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