Nanoflow Cytometric Analysis of Tumor Exosomes and Extracellular Vesicles

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Overview: Extracellular Vesicles (EVs) are continuously released, carry surface receptors and RNA/DNA cargo related to the state of their cell of origin, and are known to cross from the tumor microenvironment into the blood and into the urine. Therefore, EVs have tremendous potential as non-invasive biomarkers for diagnosis, risk-stratification, treatment selection, and treatment monitoring. The Jones Lab has developed a first-in-class pipeline to characterize EV heterogeneity and provide high sensitivity quantification of informative EV subsets by combining multiplex assays with high resolution, single EV flow cytometric methods together into a Multiplex-to-Single EV Analysis (Mt-SEA) pipeline, which is optimized for analysis of EV-associated protein markers and EV-borne RNA and DNA.

Methods: Surface proteins were interrogated with single EV flow cytometry (Morales Kastresana, et al, *Scientific Reports* 2016), and multiplex bead-based assays, RNA by HTG EdgeSeq, and DNA analysis with mutation analysis with PCR. Data analysis was performed with new software from our lab with modules for extraction of multidimensional flow cytometry data, heat map formation, hierarchical clustering, principal components analysis, and other advanced analytical tools for the Jones Lab's Mt-SEA pipeline.

Results / Conclusions: By combining multiplex assays with high-resolution, single EV flow cytometric methods together into a Multiplex-to-Single EV Analysis (Mt-SEA) pipeline, we are able to characterize a broad range of EV subsets, while also measuring the concentration of specific EV populations and their DNA cargo. Our pipeline especially allows sorting for subset studies, and we find that miRNA RNAseq with as few as one million EVs demonstrate prostate cancer miRNA signatures in sorted PSMA+ EVs. Thus, it may be possible to interrogate tumor EV subset signatures in a patient's peripheral EVs (isolated from clinical biofluids such as plasma and serum), without requiring that the patient undergo a direct biopsy of the tumor.