Characterization of E0771 Exosomes

CNF Project Number: 2780-19
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Primary Source(s) of Research Funding: PSOC Pilot Project Funding
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Primary CNF Tools Used: NanoSight

Abstract:
Pathologic activation of the blood clotting system in cancer is associated with systemic thrombotic events as well as transformation, growth and metastasis of various tumors [1,2]. Coagulation is activated primarily by tissue factor (TF). TF is overexpressed in breast tumors in situ and in breast cancer cell lines, particularly triple negative cells [3,4]. Overexpression of TF in patient tumors correlates with a poor prognosis [3]. Cancer cells and the tumor microenvironment induce a protumorigenic, pro-angiogenic, and immunosuppressive phenotype in tumor-associated immune cells like macrophages [9]. It is unknown whether breast cancer cell-generated TF-coagulation complexes and PARs regulate macrophage recruitment to tumors or whether they subsequently modulate macrophage behavior in tumors. This is important since macrophage recruitment and regulation contributes to angiogenesis, metastasis and tumor progression [10-12].

We hypothesized that breast cancer-associated hemostatic components regulate macrophage recruitment and their inflammatory, angiogenic and hemostatic activity. To investigate this question, we determined that cancer-derived extracellular vesicles had intrinsic procoagulant activity and conferred that procoagulant activity to macrophages. A key part of our work was quality control of these extracellular vesicles that we subsequently used in our macrophage experiments. We used the NanoSight particle analyzer to characterize the extracellular vesicle populations purified from cancer cell-conditioned and control media. Data obtained using the NanoSight confirmed that we isolated particles 100-200 nm, compatible with extracellular vesicles. Altogether, our data show that breast cancer-derived microparticles confer procoagulant activity to macrophages, which may play a key role in the connection between coagulation and inflammation to regulate tumor growth and anti-tumor immunity.

Summary of Research:
In this project, we used the CNF NanoSight to perform quality control on our cancer-derived and control extracellular vesicles that were then used in additional experiments. Because of the NanoSight data, we demonstrated the procoagulant activity of a mouse breast cancer cell line and found that the vesicles derived from the cells accelerated clotting in mouse plasma.

Overnight incubation of a mouse macrophage cell line with the isolated vesicle fraction from tumor-conditioned, but not cell-free, media increased the procoagulant activity of the mouse macrophage cell line. This supported our hypothesis that tumor cells upregulate procoagulant activity in macrophages. Our goal with using the Cornell NanoScale Facility was to characterize the size distribution of the obtained vesicles.

We found that the microvesicle fraction consisted of a dominant population of particles 100-200 nm, supporting successful isolation of various subsets of extracellular vesicles shed from tumor cells (Figure 1). These data supported several grant applications currently under review.
References:


Figure 1: A) Schematic for nanoparticle isolation and analysis using the CNF NanoSight. Mouse mammary cancer cells (E0771) were cultured to produce extracellular vesicles (EV) in Opti-Mem media. Vesicles were isolated by differential centrifugation and subjected to NanoSight particle tracking analysis. B) Example NanoSight results of E0771 EV compared to media only EV preparations. These data demonstrate isolation of appropriate size EVs from cell cultures and not from media controls. These preparations were used in follow on experiments.