Characterization of Cancer Microvesicles

CNF Project Number: 2780-19
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Primary Source(s) of Research Funding: Physical Sciences Oncology Center Pilot Project
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Primary CNF Tools Used: Malvern NS300 Nanosight

Abstract:
Pathologic activation of hemostasis in cancer is associated with systemic thrombotic events and transformation, growth and metastasis of various tumors [1,2]. Tissue factor (TF), the main activator of coagulation, is over-expressed in breast tumors in situ and in breast cancer cell lines, particularly triple negative cells [3,4] and expression in patient tumors is correlated with a poor prognosis [3]. Cancer-associated TF produces coagulation factor complexes that trigger thrombosis and induce cell signaling via protease-activated receptors (PARs). Macrophages are recruited from bone marrow-derived cells and blood monocytes and play key roles in pathologic hemostasis. Exposure to cancer cells and the tumor microenvironment induces a protumorigenic, pro-angiogenic, and immunosuppressive phenotype in tumor-associated macrophages [9]. However, 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].

Introduction:
We hypothesize that breast cancer-associated hemostatic components regulate macrophage recruitment and their inflammatory, angiogenic and hemostatic activity. To investigate this question, we have demonstrated that conditioned media from a mouse breast cancer cell line enhances procoagulant activity of mouse macrophages. To determine the active component of the conditioned media, we isolated the microvesicles by ultracentrifugation and showed they had intrinsic procoagulant activity and conferred procoagulant activity to macrophages. We used the Nanosight NS300 to characterize the microvesicle populations purified from cancer cell-conditioned and control media.

Data obtained using the Nanosight NS300 confirmed that we isolated particles < 100 nm, compatible with exosomes. 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 tested the procoagulant activity of a mouse breast cancer cell line and found that the cells accelerated clotting in mouse plasma. We isolated the microvesicle fraction shed from the mouse breast cancer cell line into conditioned media using ultracentrifugation and tested the procoagulant activity of the isolated microvesicle fraction. We found that the microvesicle fraction also demonstrated procoagulant activity. Overnight incubation of a mouse macrophage cell line with the isolated microvesicle fraction from tumor-conditioned, but not cell-free, media increased the procoagulant activity of the mouse macrophage cell line. This supports 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 microparticles, using cell-free media as a negative control. For this we used the Nanosight NS300 instrument. We found that the microvesicle fraction consisted of a dominant population of particles < 100 nm, supporting successful isolation of exosomes shed from tumor cells (Figure 1).
References:


