Characterization of Cancer Microvesicles

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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).

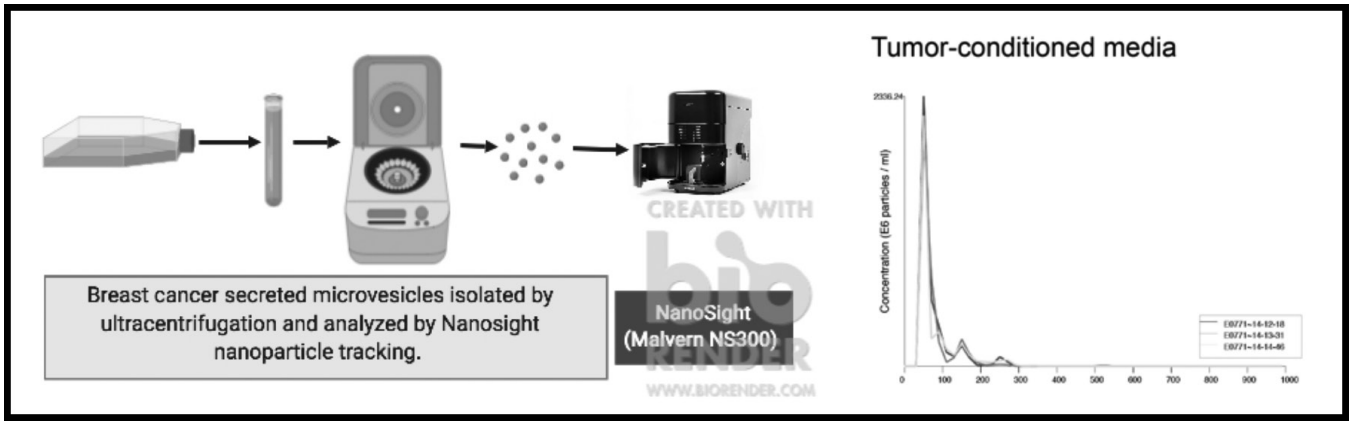

Figure 1: Schematic for nanoparticle isolation and analysis using the Nanosight. Example results of nanoparticle tracking analysis of microvesicles isolated by ultracentifugation from mouse breast cancer cell tumor-conditioned media. A high concentration of particles < 100 nm, compatible with exosomes, was found in the tumor-conditioned media preparation.

References:

[1] Khan UT, Walker AJ, Baig S, et al. Venous thromboembolism and mortality in breast cancer: cohort study with systematic review and meta-analysis. BMC Cancer 2017;17:747.

[2] Cole M, Bromberg M. Tissue factor as a novel target for treatment of breast cancer. Oncologist 2013;18:14-18.

[3] Vrana JA, Stang MT, Grande JP, et al. Expression of tissue factor in tumor stroma correlates with progression to invasive human breast cancer: paracrine regulation by carcinoma cell-derived members of the transforming growth factor beta family. Cancer Res 1996;56:5063-70.

[4] Che SPY, Park JY, Stokol T. Tissue Factor-Expressing Tumor-Derived Extracellular Vesicles Activate Quiescent Endothelial Cells via Protease-Activated Receptor-1. Front Oncol 2017;7:261.

[5] Versteeg HH, Schaffner F, Kerver M, et al. Inhibition of tissue factor signaling suppresses tumor growth. Blood 2008;111:190-9.

[6] Bourcy M, Suarez-Carmona M, Lambert J, et al. Tissue Factor Induced by Epithelial-Mesenchymal Transition Triggers a Procoagulant State That Drives Metastasis of Circulating Tumor Cells. Cancer Res 2016;76:4270-4282.

[7] Palumbo JS, Talmage KE, Massari JV, et al. Tumor cell-associated tissue factor and circulating hemostatic factors cooperate to increase metastatic potential through natural killer cell-dependent and -independent mechanisms. Blood 2007;110:133-141.

[8] Eftekhari R, de Lima SG, Liu Y, et al. Microenvironment proteinases, proteinase-activated receptor regulation, cancer and inflammation. Biol Chem 2018;399:1023-1039.

[9] Aras S, Zaidi MR. TAMeless traitors: macrophages in cancer progression and metastasis. Br J Cancer 2017;117:1583-1591.

[10] Gil-Bernabé AM, Ferjancic S, Tlalka M, et al. Recruitment of monocytes/macrophages by tissue factor-mediated coagulation is essential for metastatic cell survival and premetastatic niche establishment in mice. Blood 2012;119:3164-3175.

[11] Lin L, Chen Y-S, Yao Y-D, et al. CCL18 from tumor-associated macrophages promotes angiogenesis in breast cancer. Oncotarget 2015;6:34758-34773.

[12] Su S, Liu Q, Chen J, et al. A positive feedback loop between mesenchymal-like cancer cells and macrophages is essential to breast cancer metastasis. Cancer Cell 2014;25:605-620.