Mimicking Platelet-Cancer Cell Interactions for Targeted Drug Delivery in Metastatic Breast Cancer

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Statement of Purpose: Tumor metastasis occurs when cancer cells dislodge from a primary tumor and migrate to other sites in the body to form secondary colonies. This migration can occur through the lymphatic system as well as through the vasculature. Regarding cancer cell migration through the vascular system, there is compelling evidence that blood platelets, beyond their traditional roles in hemostasis, have a major contribution. For example, platelets can (i) facilitate the intravasion of tumor cells from primary cancer site into the vasculature by secretion of various chemokines, cytokines, and proteases, (ii) bind to circulating cancer cells to form a microthrmbi "cloak" thereby preventing the surveillance and destruction of the cancer cells by the immune system, (iii) facilitate adhesion and arrest of the circulating cancer cells at the vascular wall at a distal site under a physiological hemodynamic flow environment, (iv) facilitate extravasation and inflammatory microenvironment development at the secondary site for the cancer cells to invade and form metastatic colonies [1,2]. Rationalizing from such mechanistic possibilities, we have first investigated the expression of various platelet-interactive receptors and proteins on MDA-MB-231 (pro-metastatic) versus MCF-7 (low-metastatic) human breast cancer cells, to gain insight about possible avenues of platelet-bindng. Building on this knowledge, we have studied the interaction of activated versus resting platelets with these two cancer cell types in a flow environment, *in vitro*, using a Parallel Plate Flow Chamber (PPFC). Finally, utilizing this insight, we have engineered liposome-based particle constructs that can mimic the molecular binding interactions between platelets and metastatic cancer cells, and have investigated their ability to actively target and bind to the metastatic cells. These studies can potentially lead to effective ways of delivering therapeutic and imaging agents selectively targeted to metastatic cancer.

Materials and Methods: *Identification of platelet-interactive receptors on MDA-MB-231 versus MCF-7 cancer cells:* MDA-MB-231 and MCF-7 breast cancer cell lines were cultured to ~ 85% confluence, plated onto 12-well plates and fixed with 4% paraformaldehyde (PFA). Fluorescently-tagged antibodies staining various platelet-interaction relevant receptors (GPIIb-IIIa, P-, E-, L-Selectin, $\alpha_v\beta_3$, GPIa-IIa, and GPIb-α) were then incubated with the cells. The extent of expression of various receptors on the metastatic versus non-metastatic cells was quantified by evaluating the fluorescence intensity using a plate reader and the measurements were averaged over the entire cell-covered surface of each well. *Interactions of platelets with metastatic versus non-metastatic cancer cells in vitro:* Platelets ('resting' or ADP-activated) were labeled with Calcien (green fluorescence) and were allowed to flow over a monolayer of MCF-7 or MDA-MB-231 cells (co-stained with blue DAPI for nucleus and red Phalloidin for actin) in a PPFC set-up for 30 min at 5 dynes/cm^2. The binding of platelets to cancer cells was analyzed using fluorescence imaging. *Fabrication of platelet-mimetic particles and study of their binding with metastatic versus non-metastatic cancer cells in vitro:* Fluorescently-labeled liposomal particles, surface-decorated with ligands that can mimic the platelet-cancer cell interactions, were allowed to flow over a monolayer of MCF-7 versus MDA-MB-231 cancer cells under 5 dynes/cm^2 shear stress for 30 minutes. The binding of the particles on the cancer cells was quantified using surfaced average fluorescence intensity analysis.

Results and Discussion: Our results showed significantly higher expression of several platelet interaction-promoting receptors on the MDA-MB-231(pro-metastatic) cells compared to the MCF-7 (low-metastatic) cells. In the PPFC studies, ADP-activated platelets bound significantly more to MDA-MB-231 cells compared to the MCF-7, validating the role of these receptors in platelet-metastatic cancer cell interactions (Figure 1). Mimicking these interactions with ligand-decorated liposomal constructs enabled significantly enhanced binding to the metastatic cells compared to the non-metastatic cells (Figure 1). These studies can lead to effective ways of metastasis-targeted drug delivery.

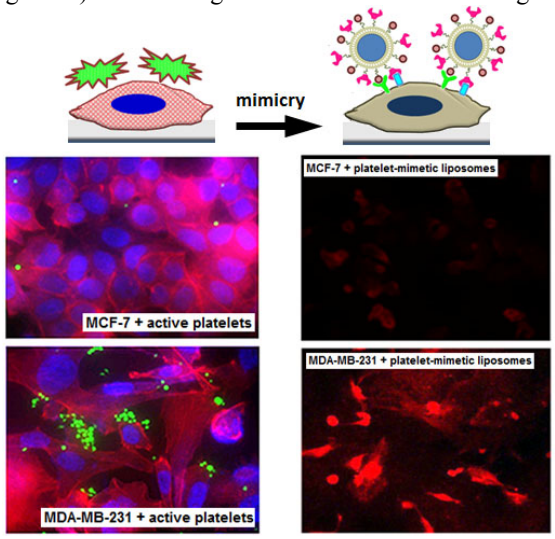

Figure 1: Interaction of low metastatic potential (MCF-7) and high metastatic potential (MDA-MB-231) cells with active platelets and platelet-mimetic liposomes under flow.

Conclusions: Hematologic pathways of metastasis are largely facilitated by active platelets. These mechanisms can be exploited using platelet-mimetic particles for creating metastasis-targeted drug delivery systems.

References:

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2. L.J. Gay, B. F. Habermann, "*Contribution of platelets to tumour metastasis*." **Nature Reviews Cancer**, 2011; 11: 123-13