Platelet Inspired Liposomes for Delivery of Doxorubicin to 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 often occurs through the vasculature (hematologic metastasis).To this end, there is compelling evidence that beyond their traditional roles in hemostasis and thrombosis, blood platelets have a major contribution to metastatic cell trafficking and pro-metastatic signaling [1, 2]. For example, active platelets can (i) facilitate epithelial–to-mesenchymal transformation and intravasation of cancer cells from primary tumor, (ii) can bind to these circulating tumor cells (CTCs) to form a microthrombi "cloak" that allows the CTCs to avoid immune surveillance, (iii) can aid in adhesion and arrest of the CTCs at the vascular wall at a distal site under blood flow, and (iv) can promote extravasation of CTCs and metastatic microenvironment development at secondary site [1,2]. Rationalizing from such mechanisms, we have investigated the possibility of utilizing platelet-inspired ligand-based interactions to actively target metastatic cells for drug delivery. For this, we have compared the expression of platelet-interactive receptors on MDA-MB-231 (pro-metastatic) versus MCF-7 (low-metastatic) human breast cancer cells, and have also investigated the binding of these cells to active versus resting human platelets under flow [3]. Building on these studies, we have engineered liposomal vehicle constructs that can mimic the relevant molecular binding interactions between active platelets and metastatic cancer cells. We have investigated their ability to actively bind the MDA-MB-231 cells compared to MCF-7 cells. We have further investigated the loading and targeted delivery of a model anti-tumor drug Doxorubicin to these cells and have evaluated the resultant cell-killing efficacy.

Materials and Methods: *Identification of platelet-interactive receptors on MDA-MB-231 versus MCF-7 cells:* The two cancer cell lines were cultured to ~ 85% confluence, fixed with 4% paraformaldehyde (PFA) and subjected to immunofluorescent staining for various platelet-interactive receptors, specifically, GPIIb-IIIa, P-, E-, L-Selectin, $\alpha_v\beta_3$, GPIa-IIa, and GPIb-α. The corresponding expression levels were quantified by evaluating the fluorescence intensity using a plate reader.

Fabrication of platelet-inspired liposomes and analysis of their interaction with cancer cells: Fluorescently-labeled liposomes, surface-decorated with ligands that can mimic the platelet-cancer cell interactions, were allowed to flow over a monolayer of MCF-7 or 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 fluorescence intensity analysis.

Encapsulation of DOX into platelet-inspired liposomes: Platelet-inspired liposomes were suspended in PBS with adjusted pH of 10.5. DOX dissolved in PBS with pH of 7 was heated to 65 °C and combined with the liposome solution. DOX was loaded into the liposomes driven by the pH gradient, and encapsulation efficiency (EE) was determined by UV-visible spectroscopy. Subsequently, DOX release kinetics was studied at pH 7.4 and 6.5 using dialysis technique by monitoring the released drug concentrations with UV-visible spectroscopy.

Targeted delivery of DOX to metastatic cells using platelet-inspired liposomes and resultant cell killing. The MCF-7 and MDA-MB-231 cells were grown to ~80% confluence in culture and incubated with the DOX-loaded platelet-inspired liposome suspension (added to media) in an incubator for 1 hour at 37 °C with gentle shaking. Following incubation, the liposome-added media was removed, and the resultant effect of Doxorubicin delivery and cell killing was examined using standard MTT assay 48 hours after completion of liposome incubation time.

Results and Discussion: MDA-MB-231 cells showed significantly enhanced expression of platelet-interactive receptors compared to MCF-7 cells. Based on the possible interaction mechanisms, platelet-inspired liposomes were prepared that can simultaneously target β3 integrins and P-selectin. These showed significantly enhanced capability of binding to the MDA-MB-231 cells (**Figure 1**) compared to MCF-7 cells. DOX EE in these liposomes was determined to be ~90% and DOX release folowed first order kinetics. Incubation of the DOX-loaded platelet-inspired liposomes with the cancer cells showed enhanced binding and uptake of DOX in the pro-metastatic cells compared to low-metastatic cells within shorter incubation times. This resulted in significantly higher killing of the MDA-MB-231 cells (**Figure 2**) compared to DOX delivery by non-targeted liposomes .

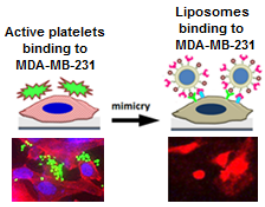

Figure 1. Targeting of platelet-inspired liposomes to MDA-MB-231 cells

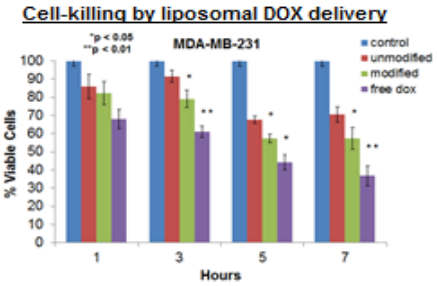

Figure 2. Targeted DOX delivery by liposomes and resultant cell killing

Conclusions: The results demonstrate the feasibility of utilizing platelet-inspired mechanisms for enhanced active targeting of liposomal systems to metastatic cancer cells.

References:

1. B. F. Habermann, Haemostasis. 2001; 31:55-58.
2. L.J. Gay, Nat Rev Cancer, 2011; 11: 123-13
3. Modery-Pawlowski, CL et al. Biomacromolecules.
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