Characterizing the Role of Tumor-Derived Extracellular Vesicles in Breast Cancer

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Primary CNF Tools Used: Malvern NS300 Nanosight

Abstract:

Breast cancer frequently metastasizes to bone, leading to osteolytic bone degradation and poor clinical prognosis. Therapeutic options are largely ineffective as the mechanisms underlying this process remain unclear. Increasing evidence suggests that primary tumors release soluble factors and extracellular vesicles (EVs) that can systemically prime distant organs for eventual metastasis. We have previously shown that primary breast tumors can alter bone materials properties even prior to secondary tumor formation, suggesting possible interference with bone mineralization pathways [1]. However, it remains unclear whether EVs directly contribute to alterations in the bone microenvironment prior to metastasis, whether their generation depends on tumor malignancy, and whether these vesicles differ in content and function. Our project investigates the connections between EV generation, breast cancer malignancy, the functional effect of EVs on stromal cells present in metastatic sites such as the bone.

Summary of Research:

Tumor-derived EVs, such as microvesicles (0.2-2 μm in size) shed from the plasma membrane and exosomes (30-100 μm) derived from multi-vesicular bodies, are emerging as critical yet distinct mediators of cell-cell communication in cancer. We are exploring the role of EVs in breast cancer metastasis to bone by isolating EVs from a panel of breast cancer cell lines representing varying degrees of malignancy to 1) characterize the amount and size distribution of EVs generated, and 2) evaluate their interaction with bone-mimetic microenvironments, and 3) evaluate their effects on stromal cells present in the bone microenvironment. By studying these phenomena, we hope to identify novel insights on bone metastasis progression that may inform the development of more effective treatments.

To investigate these questions, we have begun by culturing breast cancer cell lines of varying malignant potential and collecting the EVs shed by these cell lines. We are using the Nanosight instrument to analyze the size distributions and measure concentrations of EVs shed by these cell lines.

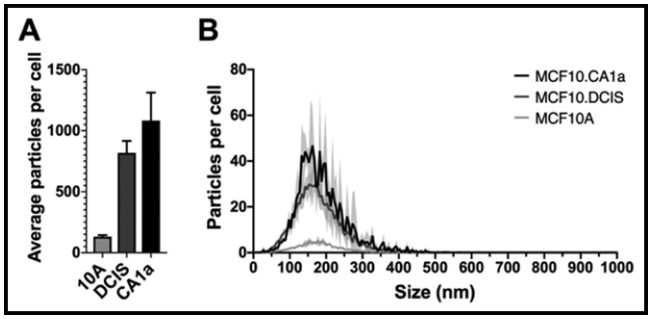

Figure 1: EV concentration and size distributions as measured by Nanosight. (a) Average number of EVs shed per cell increases with breast cancer cell malignancy potential (in increasing order: MCF10A, MCF10. DCIS, MCF10.CA1a). (b) Histograms of EV size distribution indicate increasing amounts of both microvesicles and exosomes shed by cells of increasing malignancy potential.

Our findings in Figure 1 indicate that compared to their benign counterparts, cell lines that represent more invasive and metastatic potential shed a greater amount of EVs per cell, with increases in both microvesicles and exosomes.

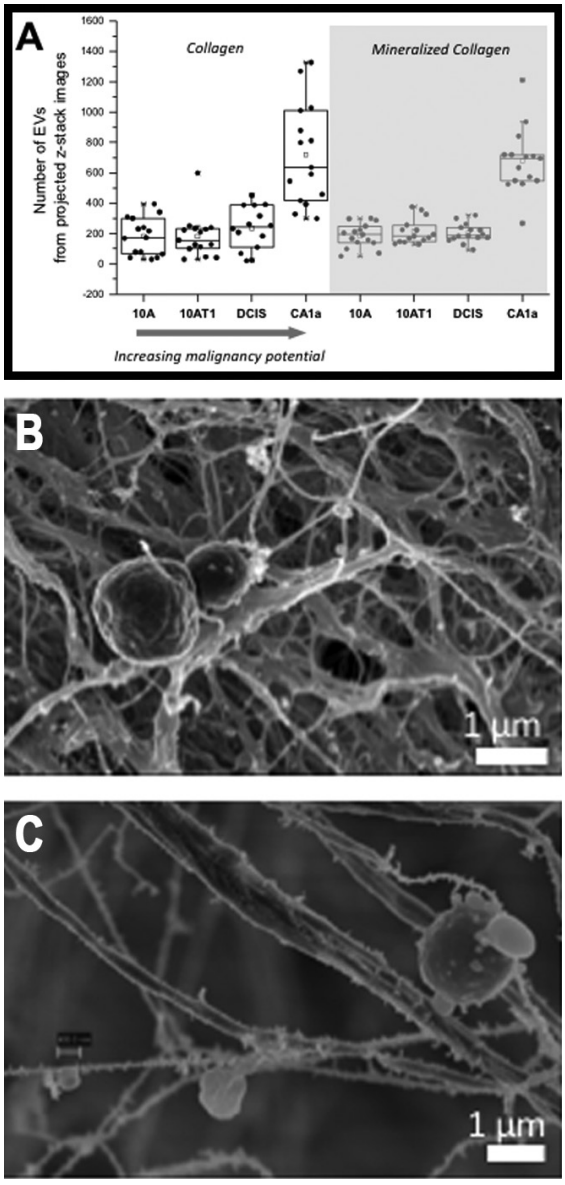

Figure 2: EVs within non-mineralized and mineralized collagen scaffolds analyzed via confocal imaging and SEM. (a) EVs shed from more malignant breast cancer cells (MCF10CA1a) show better binding ability than those shed from less malignant cells both in microenvironments. (b,c) Representative SEM images of MCF10CA1a cell-shed EVs bound to (b) collagen fibrils and (c) mineralized collagen fibrils.

Next, we have also labelled these EV populations and incubated them into collagen scaffolds with and without mineralization to examine their interactions with bone-mimetic microenvironments. As shown in Figure 2, we have imaged these EVs via confocal microscopy and found that the number of EVs bound to these bone-mimetic matrices was 3-8 fold greater when derived from more malignant cells compared to less malignant cells. Using scanning electron microscopy (SEM), we can visualize EVs from malignant cells bound to the non-mineralized and mineralized collagen fibrils in the bone-mimetic microenvironments.

These findings suggest that tumor-shed EVs, in particular those from more malignant tumor cells, can effectively bind to components of the bone microenvironment, and thus may play a role in priming the bone for subsequent metastasis. We are pursuing further investigation of the mechanisms by which these EVs bind to the bone microenvironment, as well as the effects of these EVs on bone-resident stromal cells and tumor cells within bone-mimetic microenvironments.

References:

[1] He F, Chiou AE, Loh HC, Lynch ME, Seo BR, Song YH, Lee MJ, Hoerth R, Bortel E, Willie B, Duda G, Estroff LA, Masic A, Wagermaier W, Fratzl P, Fischbach C. Multiscale characterization of the mineral phase at skeletal sites of breast cancer metastasis. Proceedings of the National Academy of Sciences, 114(40), 10542-10547. https://doi.org/10.1073/pnas.1708161114 (2017).