Evaluating 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 where it leads to osteolytic bone degradation and poor clinical prognosis. Nevertheless, therapeutic options to interfere with this process are scarce as the underlying mechanisms remain unclear. Previous studies showed that primary breast cancer tumors can alter bone materials properties and bone metastasis even prior to secondary tumor formation, suggesting possible interference with bone mineralization pathways [1]. It is well-known that extracellular vesicles (EVs) shed from tumor cells enter circulation and act as satellites of information transfer among cells. It is unknown, however, if circulating breast tumor-shed EVs contribute to early-stage changes of bone microenvironment. Our project investigates the connections between EV generation, breast cancer malignancy, the binding affinity of EVs in metastatic sites such as the bone.

Summary of Research:

We investigated how tumor-shed EVs interact with a bone-mimetic microenvironment, specifically mineralized collagen fibrils with poorly crystalline apatite crystals, and if EVs bound to the bone-like matrix can promote tumor cell binding to the bone extracellular matrix (ECM). To study interaction between tumor-shed EVs and bone ECM *in vitro*, we prepared bone-mimetic scaffolds composed of mineralized collagen type I fibrils fabricated using a polymer-induced liquid-precursor process.

We used the CNF NanoSight instrument to analyze the size distributions and measure concentrations of EVs shed by breast cancer cell lines. 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.

Next, we loaded labelled tumor-shed EVs into the scaffolds and examined the degree of binding of EVs to the collagen fibrils using confocal microscopy and SEM. The distribution and degree of binding of EVs in the matrix were correlated with various factors including breast tumor cell malignancy, the presence of mineral, and size of EVs. Breast-tumor shed EVs successfully adhered to the bone matrix via ligand-receptor interactions where the degree of binding was dependent upon tumor cell malignancy and vesicle size.

EVs adhered slightly better on non-mineralized scaffolds than mineralized scaffolds but the difference was nonsignificant. EVs shed from more malignant breast tumor cells were more effective in binding by 3-8 times to the matrix than those from less malignant cells as shown in Figure 2.

Conclusions and Future Steps:

The adhesion of breast tumor-shed EVs to the bone ECM is associated with their size and parent cell malignancy *in vitro*. These findings suggest a potential role for tumor-shed EVs in preparing a pre-metastatic niche within the bone ECM to which tumor cells are attracted. We anticipate that new understanding of adhesion ability of tumor-shed EVs to the bone ECM will contribute to our understanding of role of EVs in breast cancer bone metastasis.

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. Proc. of the National Academy of Sciences, 114(40), 10542-10547. https://doi.org/10.1073/pnas.1708161114 (2017).

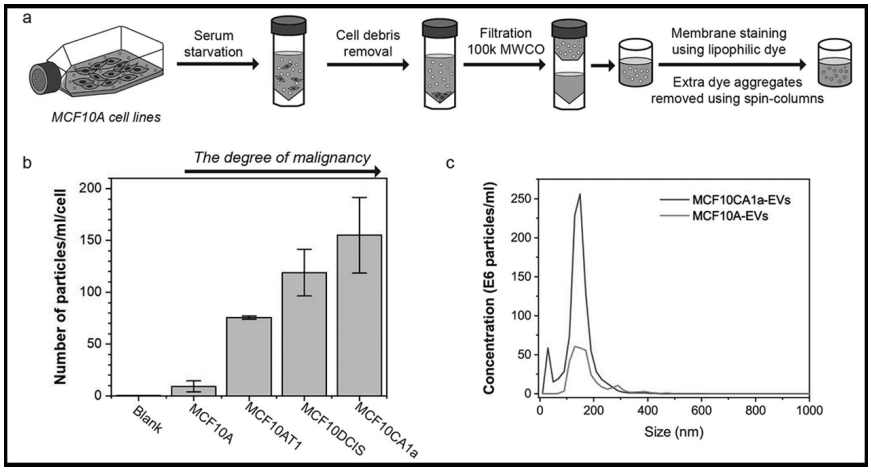

Figure 1: (a) Schematic representation of the collection of extracellular vesicles (EVs). Subconfluent MCF10A cell lines were cultured and serum-starved for 24 h. Media were collected, centrifuged to remove cell debris, and then filtered to concentrate EVs. Fluorescent lipophilic dyes were used to stain membranes of EVs, and the extra aggregates of dyes were removed using spin-columns. (b) Concentration of particles collected from the series of MCF10A cell lines as measured by NanoSight. (c) Size distribution of particles shed from MCF10A cells and MCF10CA1a cells as measured by NanoSight.

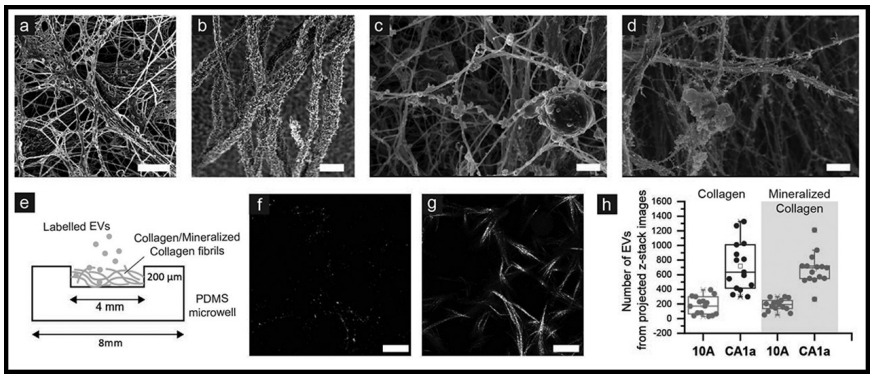

Figure 2: Binding of EVs onto 3D bone-mimetic scaffolds. (a-d) Representative SEM images showing the microstructure of (a) collagen, (b) mineralized collagen prepared via a polymer-induced liquid-precursor process, and MCF10CA1a-shed EVs bound onto (c) collagen fibrils, and (d) mineralized collagen fibrils. Scale bars = 10 µm. (e) Schematic of the bone-mimetic scaffolds used in this study. Collagen or mineralized collagen fibrils were cast into poly(dimethylsiloxane) (PDMS) microwells and fluorescently labeled EVs were incubated into the wells. (f, g) Confocal microscopy images of (f) labeled EVs in green channel and (g) collagen network in reflection mode. (h) Quantification of confocal images to compare the levels of EVs shed from MCF10A cell lines and MCF10CA1a cell lines bound onto collagen and mineralized collagen.