Mineralized Collagen Matrices Increase Breast Cancer Bone Metastatic Potential

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Statement of Purpose: Breast cancer metastasis to bone leads to worse clinical prognosis, but whether tumor cell interactions with bone extracellular matrix (ECM) play a role in this process remains unknown. Type I collagen is part of the bone ECM and thus, a relevant model ECM for studies of bone metastasis. However, type I collagen in bone primarily exists as a nanocomposite with hydroxyapatite. To test a possible functional relationship between mineralized collagen and metastatic potential of breast cancer cells, we have utilized a biomimetic approach to develop physiologically relevant bone-like collagen and have characterized breast cancer cell responses on mineralized *vs.* non-mineralized collagen.

Methods: Type I collagen fibers were cast onto coverslips and mineralized in solutions containing calcium and phosphate ions with polyaspartic acid (PAA) (Fig. a). The resulting fibrillar matrices were characterized by FT-IR (data not shown), SEM and TEM. Human MDA-MB231 breast cancer cells were cultured on these substrates and their adhesive behavior and subsequent ability to form colonies under non-adherent conditions were measured by SEM/confocal/western blot (WB)/single cell force spectroscopy (SCFS) and soft agar assay, respectively. Additionally, a GFP reporter cell line was used to detect activation of Nanog, a canonical self-renewal transcription factor of stem cells [1]. Tissue culture polystyrene (TCPS) and non-mineralized collagen were used as controls.

Results: TEM analysis indicated that PAA induced mineralization resulted in bone-like intrafibrillar mineralization of collagen (Fig. b inset). Breast cancer cells that adhered onto mineralized collagen exhibited rounder morphologies and reduced focal adhesion kinase (FAK) and phosphorylated focal adhesion kinase (pFAK) relative to those seeded on TCPS and non-mineralized collagen (Fig. b, c and d). Accordingly, the adhesion strength of cells interacting with mineralized *vs.* non-mineralized collagen was reduced (Fig e). Indeed, tumor cells proliferated less on mineralized *vs.* non-mineralized collagen (data not shown), while an opposite trend was observed for Nanog expression (Fig. f). To confirm that culture on mineralized collagen may promote MDA-MB231 metastatic and stem cell potential, colony formation following pre-culture on the different matrices was assessed [2]. Cells pre-cultured on mineralized collagen formed more colonies relative to all other groups (Fig g). Collectively, these results suggest that mineralized collagen impacts the physical interactions of tumor cells with the bone ECM and that these changes lead to differences in adhesion and proliferation as well as stem cell properties, all conditions playing a critical role in the initiation and progression of bone metastasis. Future studies will assess the functional relevance of these findings *in vivo*.

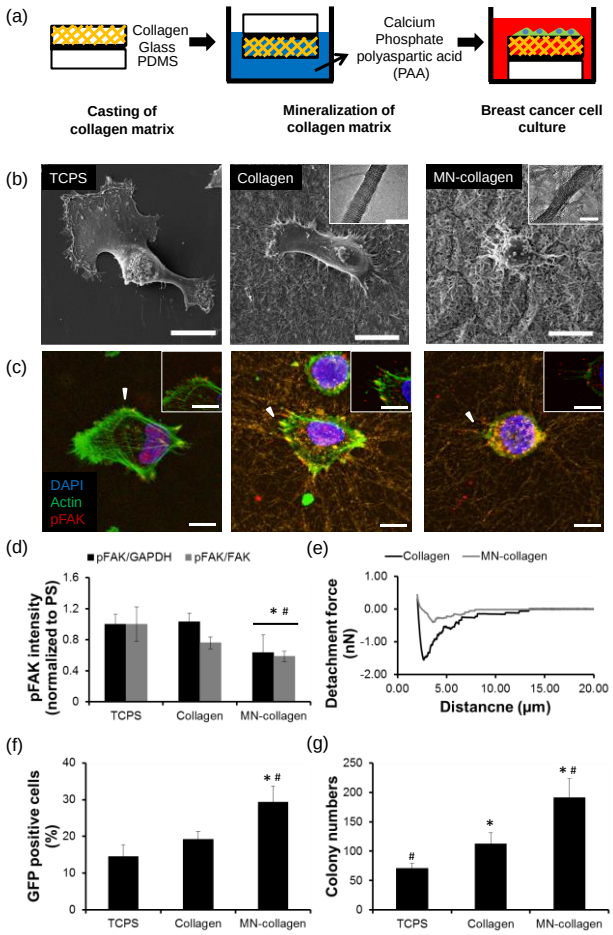

Figure. (a) Schematic of intrafibrillar mineralization of collagen. (b) Representative SEM and TEM (inset) images of cell morphology and matrix fibers (inset). (c) Representative confocal images of focal adhesion formation. Scale bar = 10 μm (SEM/confocal) and 200 nm (TEM). (d) WB analysis of focal adhesion. (e) Cell detachment force analysis by SCFS. (f) Nanog-GFP expression and (g) Colony formation in soft agar. * and # indicate significant differences ($P < 0.05$) when compared with TCPS and collagen, respectively.

Conclusions: We have utilized a biomimetic approach to develop cell culture substrates with intrafibrillar collagen mineralization for characterizing breast cancer cell interactions with the bone ECM. Our results suggest that interactions with mineralized collagen promote breast cancer cell characteristics that may regulate their stemness and thus, clinical outcome. The substrates described herein will enable future studies to gain new insights into the role of the bone ECM in breast cancer metastasis.

Reference: [1] Thiagarajan PS. Stem Cells. 2015;33: 2114-2125. [2] Seiichi M. Oncogene. 2009;28:2796-2805.