Mechanical Regulation of Breast Cancer Bone Metastasis via Osteocytes' Signaling to Endothelial Cells

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INTRODUCTION

Bone Metastases (migration of cancers to bone)

- 65-85% of patients with advance breast cancer develop bone metastasis [1].
- Significantly increase patients' mortality and morbidity with symptoms such as pathological bone fractures, pain, hypercalcaemia, and spinal cord compression syndromes.
- Metastasized cancer cells are capable of **disturbing the normal bone turnover balance**, causing bone lesions and accelerating the release of growth factors from bone matrix, which in turn aid cancer cell survival, resulting in a vicious cycle.

Osteocytes

- Major population of cells in the bone.
- **Mechanosensors** of the bone that translate mechanical loading on the bone (experienced by osteocytes as oscillatory fluid flow through the lacunae-canalicular network in which they reside) into biochemical signals and **regulate bone turnover**.
- Therefore, exercise, often suggested as an intervention for patients suffering from breast cancer, regulate bone remodeling via osteocytes and may be able to break the vicious cycle between bone lesion and tumor growth.

Endothelial Cells

- Present in large number and close proximity to the metastasizing cancer cells in blood vessels.
- On top of being the first barrier between metastasizing cancer cells and the bone, they also **play a major role in various processes in metastasis**, such as adhesion of cancer cells, angiogenesis to provide nutrients and migration paths to tumor, and signalling directly to cancer cells.
- Respond to osteocyte signalling .

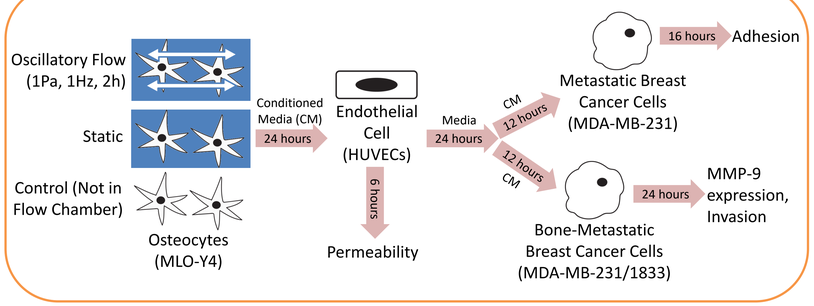
Bone Mechanical Loading and Bone Metastasis

- We previously showed that mechanically stimulated osteocytes affect breast cancer cell apoptosis and migration both directly with chemokines and indirectly through osteoclasts and endothelial cells [2]. Specifically, direct signalling from the mechanically stimulated osteocytes seem to be pro-metastatic by attracting breast cancer cells and reducing their apoptosis. Interestingly, signalling mediated by endothelial cells and osteoclasts showed opposite results and **demonstrated the anti-metastatic potential of mechanically stimulated osteocytes**.
- *In vivo* study showed that bone mechanical loading through tibial compression reduces the growth of secondary breast tumors in the bone [3].
- Osteocytes stimulated with uni-directional flow increased connexin hemichannels expression and ATP release, which reduced breast cancer cell migration [4].

HYPOTHESIS

Endothelial cells' response to mechanically stimulated osteocytes affect the metastatic potential of breast cancer cells.

EXPERIMENTAL DESIGN



METHODS

Cell Culture

- MLO-Y4 osteocyte-like cells (gift of Dr. Bonewald, Indiana University): Cultured in α-MEM (2.5% CS, 2.5% FBS, 1%P/S) on collagen-coated surface.
- Human Umbilical Vein Endothelial Cells (HUVECs) (gift of Dr. Young, University of Toronto): Cultured in endothelial cell growth media from Wisent.
- MDA-MB-231 metastatic breast cancer cells: Cultured in F-12K media (10% FBS, 1%P/S).
- MDA-MB-231/1833 bone-metastatic breast cancer cells: Cultured in DMEM (10% CS, 1% P/S).

Mechanical Loading through Oscillatory Fluid Flow

- Parallel-plate flow chamber to generate sinusoidal wave (1 Pa peak shear stress, 1Hz, 2 hours).
- Conditioned media (CM) collected 24 hours post-flow.

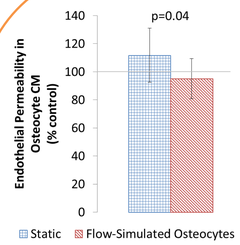
Assays

- Permeability: Fluorescein-dextran (40 kDa) was added to Transwells with confluent HUVECs conditioned in MLO-Y4 CM. Fluorescence was measured after 30 minutes.
- Adhesion: 30 minutes after adding cell tracker green-stained MDA-MB-231 cells to HUVECs conditioned in MLO-Y4 CM, 30 minutes of oscillatory fluid flow (1Pa; 1Hz) was applied.
- MMP-9 expression: qPCR.
- Invasion: Transwells were coated with 1mg/mL Matrigel. Conditioned MDA-MB-231/1833 cells were stained with cell tracker green and allowed to migrate across Matrigel towards media supplemented with 20% calf serum for 24 hours.

Statistics

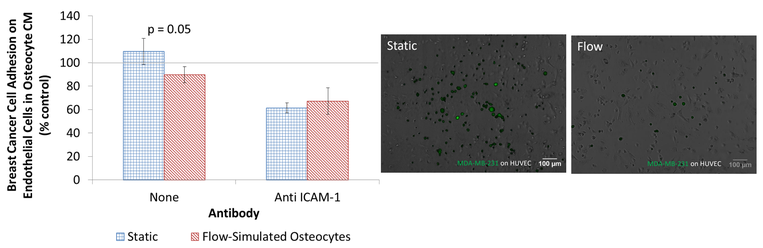
- P-values were obtained using paired Student's t-test.
- Data normalized to controls (results with CM from MLO-Y4 cells not placed in flow chambers).

RESULTS



Osteocytes → Endothelial Permeability

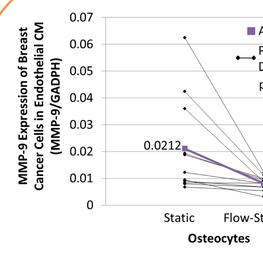
- Permeability of human umbilical vein endothelial cells (HUVECs) is 15% lower in conditioned media (CM) from flow-stimulated MLO-Y4 osteocytes.
- Data presented as mean ± standard deviation, normalized to controls (permeability HUVECs conditioned in CM from MLO-Y4 cells not in flow chambers), n = 3 experiments with 15 samples.
- The reduction in endothelial permeability may be due to the increase in PGE-2 (Prostaglandin E2) production by mechanically loaded osteocytes [5] as PGE-2 had been shown to enhance endothelial layer [6].



Osteocytes → Endothelial → Breast Cancer Cell Adhesion

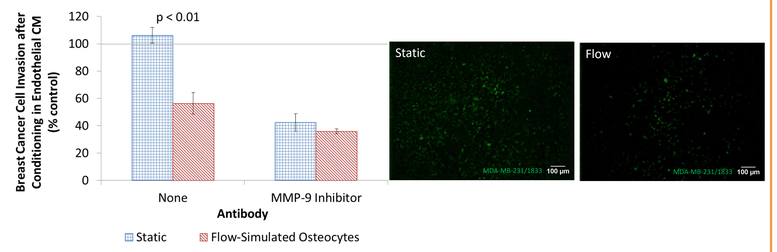
- Adhesion of metastatic breast cancer MDA-MB-231 cells to human umbilical vein endothelial cells (HUVECs) is 18% lower when HUVECs were conditioned in conditioned media (CM) from flow-stimulated MLO-Y4 osteocytes. Data is presented as mean ± standard deviation, normalized to control (adhesion to HUVECs conditioned in CM from MLO-Y4 cells not in flow chambers), n = 3 experiments with 15 samples.
- Application of anti-ICAM-1 (Intercellular Adhesion Molecule 1; expression affected by osteocytes [7]) antibody abolished the difference. Data is normalized to control (adhesion to HUVECs conditioned in CM from MLO-Y4 cells not in flow chambers), n = 3 experiments with 14 samples.

RESULTS



Osteocytes → Endothelial → Breast Cancer Cell MMP-9

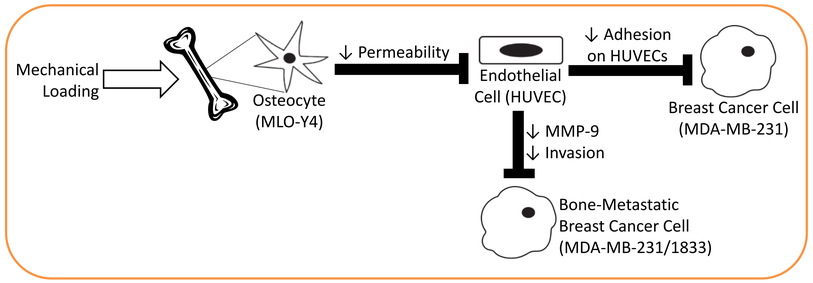
- MMP-9 (matrix metallopeptidase 9; degrades collagen) is important for cancer cells to degrade matrix during invasion into the bone and, in addition, promotes trans-endothelial migration [8].
- MMP-9 expression by MDA-MB-231/1833 bone-metastatic breast cancer cells is 62% lower when MLO-Y4 osteocytes are stimulated with flow.
- Data is normalized to expression of GADPH (glyceraldehyde 3-phosphate dehydrogenase; house-keeping gene), n = 11 from 3 experiments.



Osteocytes → Endothelial → Breast Cancer Cell Invasion

- Invasion of MDA-MB-231/1833 bone-metastatic breast cancer cells after conditioning in conditioned media (CM) from human umbilical vein endothelial cells (HUVECs) is 47% lower when HUVECs were conditioned in CM from flow-stimulated MLO-Y4 osteocytes. Data is presented as mean ± standard deviation, normalized to control (invasion when MLO-Y4 cells were not placed in flow chambers), n = 3 experiments with 18 samples.
- Application of MMP-9 inhibitor abolished the difference. Data is normalized to control (invasion when MLO-Y4 cells were not placed in flow chambers), n = 2 experiments with 10 samples.

SUMMARY



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