Osteocytes' VEGF Secretion in Response to Oscillatory Fluid Flow Supports Breast and Prostate Cancer Cell Migration

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INTRODUCTION

- Bone metastases (migration of cancers to bone) [1]
  - Common and severe complications of cancer with an estimate of 70% of patients with breast and prostate cancer developing bone metastasis.
  - Symptoms include pathological bone fractures, pain, hypercalcaemia, spinal cord and nerve-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 during bone resorption. The growth factors in turn enhance tumor cells growth and result in a vicious cycle. [2]
- Osteocytes, mechanical loading, and bone remodeling [3]
  - Osteocytes are **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 to other cells (such as osteoblasts and osteoclasts) and **regulate bone turnover**.
  - Therefore, exercise, often used as an intervention for patients suffering from breast cancer [4], could regulate bone remodeling via osteocytes.
- Very little is known about the potential effect of osteocytes' response to mechanical loading on the bone on the progress of bone metastasis.
- **VEGF** (vascular endothelial growth factor)
  - VEGF secretion by osteocytes has been shown to increase under mechanical loading [5].
  - VEGF has been shown increase the migration [6], invasion, and adhesion [7] of breast cancer cells (MDA-MB-231).

HYPOTHESIS

VEGF secreted by **osteocytes** in response to **mechanical loading** will affect breast and prostate **cancer cell activity** (viability, proliferation, apoptosis, and migration).

Methods

Cell Culture
- MLO-Y4 osteocyte-like cells (gift of Dr. Bonewald, UMKC): Cultured in α-MEM (2.5% CS, 2.5% FBS, 1%PS) on collagen-coated surface
- MC3T3 osteoblasts: Cultured in α-MEM (10% FBS, 1%PS) on collagen-coated surface
- MDA-MB-231 breast cancer cells: Cultured in DMEM (10% FBS, 1%PS)
- PC3 prostate cancer cells: Cultured in DMEM (10% FBS, 1%PS)

Mechanical Loading through Oscillatory Fluid Flow (OFF)
- Parallel plate flow chamber
- Sinusoidal wave: 1 Pa peak shear stress, 1Hz, 2 hours
- Cells seeded on collagen-coated glass slides 48 hours prior to flow

Cancer Cell Activity Assays
- Migration: Cell-tracker-green-stained cancer cells were placed on Transwell and allowed to migrate towards conditioned medium (CM) from mechanically stimulated MLO-Y4 cells for 6 hours. Non-migrated cells were scraped off and migrated cells were imaged and counted.
- Viability: Fixable Viability Dye eFluor 450, flow cytometry
- Proliferation: BrdU, flow cytometry
- Apoptosis: APOPercentage

METHODS

VEGF Measurement and Neutralization
- The concentrations of VEGF in the conditioned medium from MLO-Y4 cells were measured with ELISA (R&D system).
- Mouse anti-VEGF neutralizing antibody (R&D systems) was added to conditioned medium at 1μg/mL.

Statistics
- P-values were obtained using Student's t-test (2-tail; unequal variance).

RESULTS

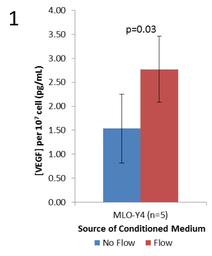


Figure 1. Concentration of VEGF is higher in CM from MLO-Y4 cells stimulated with 2-hour oscillatory fluid flow.

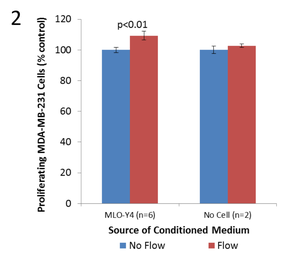


Figure 2. Proliferation of MDA-MB-231 breast cancer cells is slightly higher in CM from MLO-Y4 osteocyte-like cells stimulated with 2-hour flow.

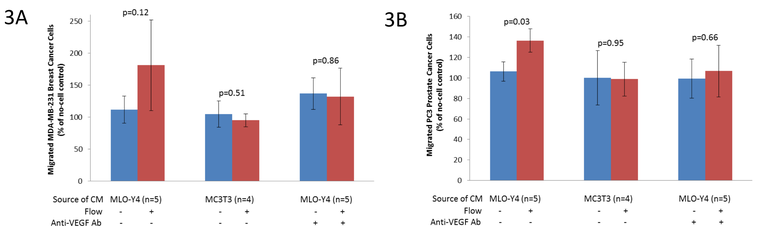


Figure 3. Migrations of **A)** MDA-MB-231 breast and **B)** PC3 prostate cancer cells are higher towards CM from MLO-Y4 cells stimulated with 2-hour flow. Anti-VEGF blocking antibody abolished the difference. Migration towards CM from MC3T3 osteoblasts showed no difference between flow and no flow. Results were normalized to cancer cell migration towards regular growth medium.

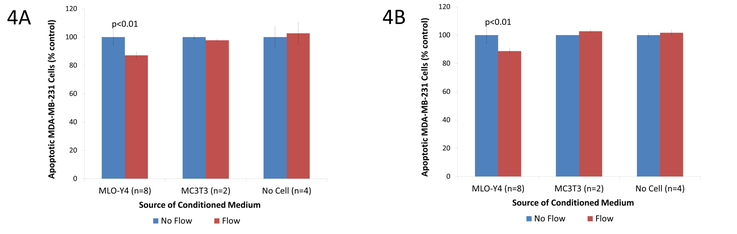


Figure 4. Percentages of apoptotic cells of **A)** MDA-MB-231 breast and **B)** PC3 prostate cancer cells are slightly lower in CM from MLO-Y4 cells stimulated with 2-hour flow. No difference was shown in CM from MC3T3 osteoblasts. Results were normalized to cancer cell apoptosis in regular growth medium.

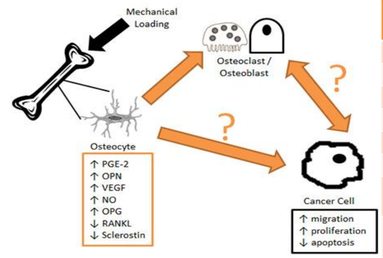
- No difference was found for cancer cells viability or PC3 proliferation.

SUMMARY

- **VEGF concentration** is higher in CM from MLO-Y4 cells stimulated with 2-hour oscillatory fluid flow (1Hz; 1Pa peak shear stress).
- CM from MLO-Y4 cells exposed to 2-hour flow **attracted** MDA-MB-231 and PC3 cells. **Anti-VEGF** neutralizing antibodies abolished the effect.
- CM from MLO-Y4 cells exposed to 2-hour flow increased MDA-MB-231 cells **proliferation** and reduced cancer cells **apoptosis** slightly.

DISCUSSION

- An *in vivo* study showed that mechanical loading through tibial compression inhibits the growth of secondary breast tumors after they metastasized to the bone [8].
  - Other cells may be involved: mechanical loading reduces osteocytes' support of osteoclastogenesis and thus osteoclasts' support of cancer cell growth and migration [9].



Osteocytes' Response to	Potential Effect on Metastases
VEGF (↑)	↑ migration (↑ CXCR-4)
Sclerostin (↓)	Higher in patients
OPG (↑)	↓ migration
RANKL (↓)	↑ migration
Dkk1 (↓)	↑ cancer growth
OPN (↑)	↑ migration
COX-2 / PGE-2 (↑)	Higher in patients
NO (↑)	↑ tumor growth (cytotoxic at high level)

- Significance: This is a novel research that provides insights into the impact of exercises on bone metastases. Examining the mechanism may also lead to potential drug targets.
- Micro-channel device will be used to incorporate osteoblasts and osteoclasts into the experimental design as their recruitment and action are strongly affected by both osteocytes and metastasized cancer cells. This allows cross-talks between different cell populations and better mimicking of the *in vivo* bone environment, while being able to investigate the mechanism cleanly without interferences and variations that exist in *in vivo* experiments.

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