

The Role of Fluid Flow on Osteocytes in the Regulation of Prostate Cancer Cell Metastatic Potential

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INTRODUCTION: Bone metastasis occurs in the vast majority (>80%) of advanced-stage prostate cancer (PCa) patients. Exercise has been recommended as an alternative cancer intervention strategy, as it has been shown to attenuate metastatic tumour progression and preserve bone structure in in vivo studies [1,2]. As major mechanosensors and regulators of the bone microenvironment, the role of osteocytes in PCa bone metastasis warrants further investigation. Previous in vitro studies have indicated that direct PCa-osteocyte interactions promote PCa cell growth and migration [3], however, these findings do not correlate with in vivo results and may be more reflective of late-stage metastatic colonization, as PCa cells must first extravasate through the vasculature and colonize the endosteal surface of bone. Thus, the aim of this study is to elucidate the role of osteocytes under mechanical loading during the early stages of the metastatic cascade in PCa bone metastasis. We hypothesize that osteocytes under mechanical loading can regulate PCa metastatic potential through other cells present in the bone microenvironment, specifically endothelial cells and osteoblasts.

METHODS: To investigate the role of osteocyte loading on PCa cell metastatic potential, both conventional flow chamber and microfluidic approaches are to be used. PCa migration, invasion, and growth in direct contact co-culture with osteoblasts were assessed. Briefly, MLO-Y4 osteocytes were placed into flow chambers and subjected to oscillatory fluid flow (OFF) at 1 Pa peak shear stress and 1 Hz for 2h. Osteocyte (OCY) conditioned media (CM) were collected 24h after loading. MC3T3-E1 pre-osteoblasts were differentiated in osteogenic medium for 10 days, then treated in a 1:1 ratio of osteogenic medium and OCY CM (static or OFF) for 72h to generate osteoblast (OB) CM. For direct contact co-cultures, MC3T3-E1s were seeded into ibidi μ -slide channels. Then, mineralized OB cultures were treated with 1:1 OCY CM and osteogenic medium for 72h. PC-3 cells were seeded into the channels and imaged daily to assess cell growth and aspect ratio (Fig 1C). Mineralized area after co-culture was determined via alizarin red staining. PC-3 adhesion to the endothelium, trans-endothelial migration in static and loading groups were assessed. Human umbilical vein endothelial cells (HUVECs) were grown to confluence in ibidi channels for adhesion experiments, or on Transwell membranes. HUVECs were then treated with OCY CM to assess PC-3 adhesion or trans-endothelial migration. For blocking experiments, HUVECs were incubated for 1.5h with vascular cellular adhesion molecule 1 (VCAM-1) blocking antibody at 2 μ g/mL. Statistical analyses were performed using paired, two-tailed t-tests or a one- or two-way ANOVA followed by a post-hoc Tukey test.

RESULTS: Wound healing migration and invasion assays indicate that OCY loading can indirectly reduce PC-3 migration (Fig 1A, $p < 0.05$) and invasion (Fig 1B, $p < 0.01$). Interestingly, PC-3 cell growth on mineralized MC3T3-E1 cells was reduced (Fig 1D, $p < 0.05$) over 72h of growth in OFF groups. Moreover, the aspect ratio of cells between PC-3s cultured on mineral matrix conditioned with OFF-stimulated osteocyte CM was reduced compared to static conditioned matrices on Days 1 and 2 (Fig 1E, $p < 0.001$, and $p < 0.01$ respectively). We also observed a reduction in mineralized area in static co-cultures (Fig 2A and B, $p < 0.05$). So far, both adhesion to HUVECs (Fig 3B, $p < 0.001$) and trans-endothelial migration (Fig 3C, $p < 0.01$) were attenuated in OFF groups. Immunofluorescence staining also revealed a decrease in VCAM-1 expression in HUVECs in OFF groups (Fig 3A), and VCAM-1 blocking experiments demonstrated neutralization of PC-3 adhesion to HUVEC monolayers (Fig 1B, not significant).

DISCUSSION: Data suggest that OCY loading can attenuate PCa migratory and invasive properties through OBs, implying a less invasive phenotype in OFF groups. Moreover, we observed a decrease in PC-3 cell growth and in cell aspect ratio in loading groups, suggesting a shift towards a more epithelial phenotype in direct contact co-cultures as well. The reduction in mineralized area in static cultures may be attributed to either lower osteogenic activity from osteocyte CM, or an increase in PC-3 MMP secretion, as PC-3s have been shown to degrade mineralized matrices through MMPs-1, 2, and 9 [4]. Enhanced MMP activity is also attributed to a more mesenchymal (invasive) cancer cell phenotype. We plan to inhibit MMPs-2 and -9 during PC-3-MC3T3 co-culture experiments to determine the cause of the reduced mineralized area. Results regarding PC-3 regulation from endothelial cells also demonstrate a reduction in metastatic potential in loading groups through adhesion and trans-endothelial migration. Blocking experiments revealed that VCAM-1, associated with inflammation and facilitating cancer adhesion/extravasation, may play a role in supporting PCa bone metastasis. Previous studies have demonstrated that osteocyte loading reduces endothelial permeability and expression of intercellular adhesion molecule-1 (ICAM-1), also associated with inflammation and metastatic cancer behaviours [5]. Based on our results, we anticipate that PC-3 extravasation on a well-established microfluidic co-culture platform will also be attenuated by osteocyte loading [6].

SIGNIFICANCE/CLINICAL RELEVANCE: This study will more provide information on the role of osteocyte loading during the early stages of PCa bone metastasis and support current theory that loading can aid in protecting bone from the vicious cycle of PCa metastasis [2]. Information from this study may be relevant for developing therapies that target osteocytes, and further supports exercise as a cancer intervention strategy to improve patient outcomes.

REFERENCES: [1] Wang et al., *Cancer Res*, 2020 [2] Arredondo, PhD Thesis, University of Sheffield, 2021 [3] Verbruggen et al., *Cancers*, 2021 [4] Sanchez-Sweatman et al., *Invasion and Metastasis*, 1998 [5] Ma et al., *J Cell Biochem*, 2018 [6] Mei et al., *Int Bio*, 2019

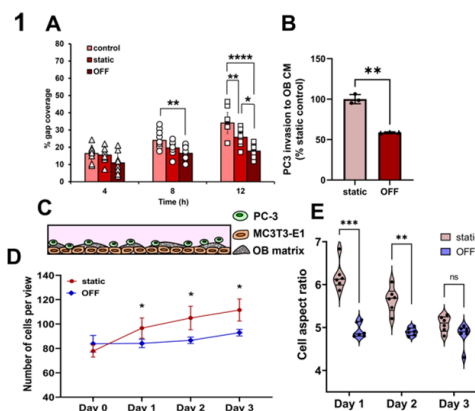


Figure 1. (A) Wound healing migration assay of PC-3 cells treated with OB CM (B) Transwell invasion assay of PC-3s towards OB CM (C) Schematic of PC-3-MC3T3 direct contact co-cultures (D) PC-3 cell growth over 72h in direct contact co-culture with OBs (E) PC-3 cell aspect ratio in direct contact co-cultures (100 cells/sample)

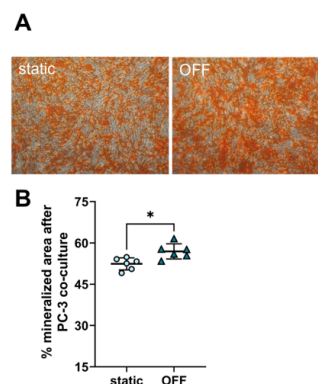


Figure 2. (A) Alizarin red stain of OBs after 72h of direct contact co-culture with PC-3 cancer cells (B) Measured mineralized area in ibidi channels from alizarin red staining

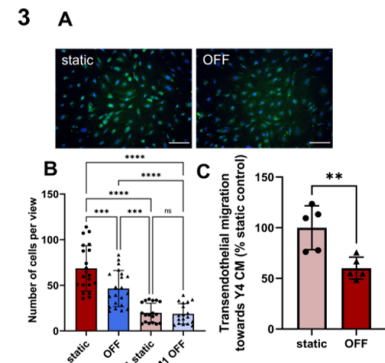


Figure 3. (A) Immunofluorescent staining of VCAM-1 in HUVEC cultures treated with static or OFF OCY CM (B) Adhesion assay of PC-3s to HUVECs in static or OFF conditions, and neutralization of adhesion via VCAM-1 blocking (C) Trans-endothelial migration of PC-3s towards OCY CM