Examining mechanobiological regulation of disseminated prostate cancer cells in the bone marrow perivascular niche

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INTRODUCTION: More than 60% of primary prostate cancer patients harbor quiescent cancer cells that have disseminated to bone marrow (BM)[1]. These disseminated cancer cells (DCCs) enter a reversible, non-dividing state, enabling metastatic recurrence several years after treatment[1]. Recent studies have identified how host cells within the BM perivascular niche, such as endothelial cells, regulate quiescence or reactivation of DCCs[2]. However, the role of mechanical forces generated through physical activity, or changes to the surrounding extracellular matrix (ECM) from aging or disease in regulating prostate cancer cell quiescence remains undefined. Therefore, the aim of this study is to examine mechanobiological regulation of prostate cancer cell quiescence within the BM microenvironment using an in vitro organotypic model of the BM perivascular niche. Specifically, we investigate how osteocytes, mechanosensitive cells in bone, regulate prostate cancer cell quiescence through the release of secreted factors upon physiological mechanical stimulation and how stiffening of the BM ECM impacts metastatic outgrowth.
METHODS: Herein, an in vitro model of microvasculature was recreated to model the perivascular niche within BM, known as microvascular niches[2,3]. BM mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (ECs) were mixed at a 5:1 ratio at a concentration of 600,000 cells/ml (Fig. 1A). 60,000 cells per well were seeded into 96-well-plates to induce organotypic vessel formation over 10 days (Fig. 1A). 400 PC-3 prostate cancer cells were seeded into each well on either Days 3, 7 or 10 during vessel formation. Once cancer cells were attached, Matrigel was introduced to co-cultures at final concentrations of either 2 or 5 mg/ml to provide a 3D environment to co-cultures and to modulate matrix stiffness. The ECM was further tuned by incorporating 0.25 or 0.5 wt% alginate crosslinked with calcium chloride to determine the role of stiffness on prostate cancer cell growth without presenting additional adhesive moieties to cancer cells. To determine the effects of mechanosensitive osteocytes on prostate cancer cell quiescence within BM microvasculature, conditioned media collected from either static or oscillatory fluid flow (OFF)-stimulated MLO-Y4 osteocytes were added to co-cultures. OFF was applied to MLO-Y4 osteocytes at a peak shear stress of 1 Pa, frequency of 1 Hz for two hours, with conditioned media collected 24 hours post-loading[4]. Static controls were included to compare with OFF-stimulated groups. Additional controls included co-cultures not treated with osteocyte conditioned media. Prostate cancer cells were cultured in BM microenvironments for another 10 days. Co-cultures were stained for CD31 (EC marker) for endpoint analyses. Statistical analyses were performed using two-tailed Student's t-tests or a one- or two-way ANOVA followed by a post-hoc Tukey test with significance taken at $\alpha = 0.05$. Each experimental group contained between n=3-5 technical replicates.
RESULTS: We demonstrate formation of organotypic EC vessels over 10 days of culture (Fig. 1B, C, **p<0.01, ***p<0.001, ****p<0.0001). Microvascular niches treated with osteocyte conditioned media over 72 hours demonstrated that OFF-stimulated osteocytes supported the formation of microvasculature (Fig. 2A, B, *p<0.05). In controls without osteocyte conditioned media, PC-3 growth was elevated in 5 mg/ml hydrogels compared to 2 mg/ml hydrogel across all timepoints, and no pattern of PC-3 growth was observed in 5 mg/ml groups when seeded at different timepoints (Fig. 2C, D, **p<0.01, ***p<0.001). In contrast, elevated PC-3 growth was observed in 2 mg/ml groups when seeded on Day 3 of vessel formation compared to later timepoints (Fig. 2C, *p<0.05). While OFF-stimulated osteocytes elevated PC-3 growth in 2 mg/ml hydrogels when seeded on Day 3, mechanical loading of osteocytes had an inhibitory effect in 5 mg/ml conditions when PC-3 cells were seeded on Days 7 or 10 (Fig. 3A, *p<0.05, **p<0.01, ns = not significant). Of interest, addition of alginate to 2 mg/ml hydrogels also resulted in elevated cancer cell growth, and mechanical loading of osteocytes also demonstrated similar inhibitory patterns of PC-3 growth observed in the stiffer 5 mg/ml hydrogels (Fig. 3B, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).
DISCUSSION: Since OFF-stimulated osteocytes promote vascularization, the enhancement of developing vasculature at early timepoints causes elevated growth of PC-3 cells in 2 mg/ml hydrogels. Elevated PC-3 growth was observed in 2 mg/ml groups when seeded on Day 3, demonstrating the role of vessel stability on PC-3 growth. As no temporal trends of PC-3 growth were observed in 5 mg/ml groups and that the addition of alginate to 2 mg/ml groups resulted in similar growth patterns observed in 5 mg/ml groups, we suspect that elevated cancer cell-ECM interactions are predominantly responsible the observed effects on PC-3 growth. The signalling pathways causing PC-3 growth in the BM niche will be determined. Future experiments will incorporate more physiologically relevant ECM molecules, such as collagen I that is typically found in BM.
SIGNIFICANCE/CLINICAL RELEVANCE: As the first study to provide preliminary evidence on mechanobiological regulation of disseminated prostate cancer outgrowth in the BM, we hope to provide insight into the role of the mechanical environment on cancer cell quiescence in BM. The findings from this study could motivate further investigation on the effects of age or disease-related alterations to BM matrix, or even the role of exercise or activation of bone anabolic pathways on cancer cell quiescence in the perivascular niche, and ultimately develop intervention strategies to modulate the proliferative status of disseminated cancer cells in BM.
REFERENCES: [1] Owen, EMBO Rep, 2020, [2] Ghajar, Nat Cell Biol, 2013 [3] Evensen, PLOS One, 2009 [4] Seaman, Adv Biol, 2025

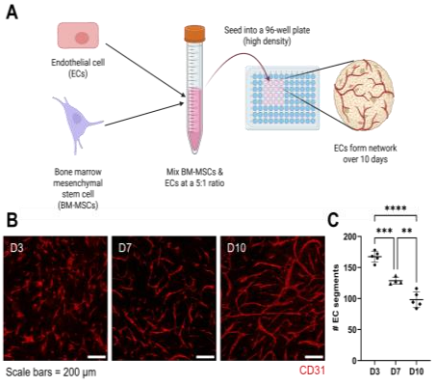

Figure 1. (A) Formation of microvascular niches. (B) Timepoint images of vessel formation over 10 days. (C) Vessel formation quantified by the number of EC segments over 10 days.

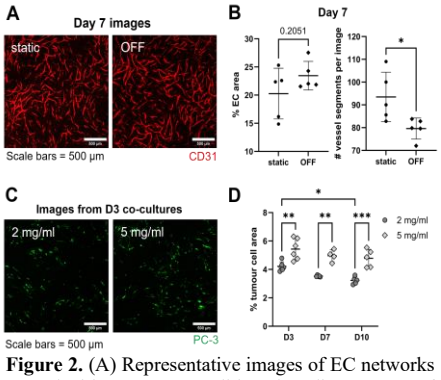

Figure 2. (A) Representative images of EC networks treated with MLO-Y4 conditioned media on Day 7 of vessel formation. (B) Quantification of EC networks in terms of %EC area and number of segments (C) Representative images of PC-3 growth in 2 mg/ml and 5 mg/ml hydrogels seeded on Day 3 of vessel formation. (D) PC-3 growth quantified as %tumour cell area when seeded on either Days 3, 7, or 10 of vessel formation in 2 or 5 mg/ml hydrogels.

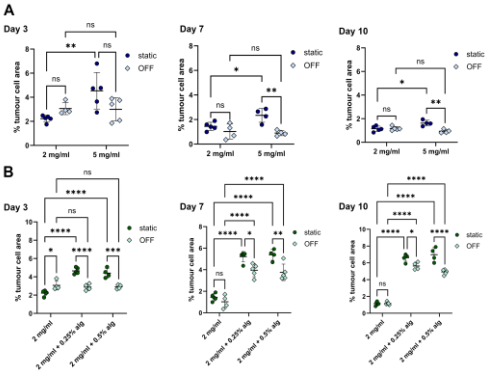

Figure 3. (A) PC-3 growth quantified as %tumour cell area in 2 or 5 mg/ml hydrogels treated with MLO-Y4 conditioned media. (B) PC-3 growth in 2 mg/ml hydrogels with 0.25 wt% or 0.5 wt% alginate.