Bidirectional Crosstalk of Resident Bone Cells and Metastatic Prostate Cancer

Max Tracy[1], Kristina Wells[1], Damian C. Genetos[1]
[1]University of California, Davis
matracy@ucdavis.edu

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INTRODUCTION: Prostate cancer (PCa) is the most prevalent form of cancer for males. While localized cases of PCa have high rates of survival, metastatic cases are characterized by higher morbidity and mortality by a large margin. PCa commonly establishes secondary tumors within bone, as the osteogenic niche provides a synergistic space for PCa to both reside in and grow. This cancer growth in bone is accompanied by excess bone growth (termed osteoblastic lesions), wherein bone resident cells and PCa will interact to direct aberrant formation of bone, further exacerbating the lesion. Our lab is particularly interested in osteocytes within this context, as these cells are known to maintain bone homeostasis, so a disruption to this balance could well implicate osteocytes in a functional role for the development of PCa lesions. Our research seeks to elucidate early paracrine interactions which attract PCa to bone, identify the role of the osteocyte in early stages of metastasis, and understand the bidirectional crosstalk between PCa and bone once PCa is established in the niche.

METHODS: To generate conditioned media, MC3T3 pre-osteoblasts, Ocy454 osteocytes, nonmetastatic LNCaP or bone metastatic C4-2B cells were grown to 80% confluence in 10% FBS media. Growth media with 1% FBS was subsequently added to each plate and cells were allowed to grow for 24 hours, before conditioned media (CM) was collected. Migration was assessed using transwell assays. Caspase 3/7 assays were used to evaluate apoptosis of osteoblasts/osteocytes due to PCa CM. One-way ANOVAs with Tukey's multiple comparisons test were used to test for significance between groups.

RESULTS: First, we used migration assays to model early recruitment of PCa to bone. Both LNCaP (a non-metastatic) and C4-2B (a bone metastatic LNCaP derivative) PCa cell lines had increased migration to MC3T3 CM compared to 1% FBS control ($n = 4$, $p < 0.05$; $n = 7$, $p < 0.05$), while Ocy454 CM saw an increase only in C4-2Bs ($n = 12$, $p < 0.05$) (**Fig A**). When directly compared, there was greater migration by C4-2B cells than LNCaP. This observation was dose dependent. To evaluate the effect of PCa on bone viability, LNCaP and C4-2B CM were applied to MC3T3s and Ocy454s. PCa CM increased caspase 3/7 activity in MC3T3 cells compared to 1% FBS media control ($n = 6$, $p < 0.05$) (**Fig B**). There was no change in Ocy454 viability by caspase3/7 assay (**Fig B**). Finally, we wanted to evaluate if the presence of PCa in bone enhanced bone cell recruitment of PCa. To model this, we used CM from MC3T3s or Ocy454s that had been previously treated with C4-2B CM. There was no difference in C4-2B migration between MC3T3 CM and PCa-pretreated MC3T3 CM (**Fig C**). However, there was increased C4-2B migration for the educated Ocy454 CM as compared to 1% FBS media ($n = 6$, $p < 0.05$), but not for Ocy454 CM compared to 1% FBS media (**Fig C**).

DISCUSSION: While previous *in vitro* work by other groups has probed the interaction of bone and PCa, there has been a relatively muted consideration for the osteocyte. Our work here shows that osteocytes do impact PCa, but to a different degree than osteoblasts, and can be educated by PCa to modulate their ongoing effect upon PCa. Apoptosis induction was also observed due to PCa soluble factors, wherein osteoblasts, but not osteocytes, saw an increase in cell death. These variable effects in both PCa attraction and cell death depending on cell type, could hint at the relative importance for these cell types to influence similar processes *in vivo*. However, this work is still limited as it is only shown *in vitro*, thus, to more completely assess osteocyte communication with PCa, a mouse model that better recapitulates the complex tumor microenvironment would add significance and represents a viable future direction.

SIGNIFICANCE/CLINICAL RELEVANCE: Metastatic prostate cancer results in low 5 year survival rates around 34% and causes varying complications that negatively affect quality of life for patients. Bone is the most common location for metastatic PCa to spread to, although our understanding of how prostate cancer interacts with the assortment of cell types here, remains incomplete; further elucidation of these interactions could provide the basis for therapeutics that seek to limit and prevent PCa spread to bone, or PCa growth in bone, to improve patient outcomes.

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IMAGES AND TABLES: