Mechanical Loading Affects Triple Negative Breast Cancer Tumor Formation in Human Bone *in vitro*

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INTRODUCTION: While mortality rates from primary tumors have decreased, the incidence of breast cancer in the United States is increasing, occurring in 1 in 8 women, with a 20-30% mortality rate associated with metastatic disease.[1,2] Bone lesions are present in 70-85% of patients who die of cancer, and are considered incurable.[2,3] Load bearing is the primary function of bone, and mechanical stimulation is essential to normal bone physiology. Cancer metastasis to bone alters the normal bone physiology, resulting in uncontrolled bone resorption and hyperplasia[5] in a vicious cycle where cancer cells stimulate bone resorption. This in turn releases biomolecules from the extracellular matrix that support cancer cell proliferation leading to additional bone destruction in a positive feedback loop.[4,6] Mechanical loading of murine tibiae inhibited tumor progression and bone destruction, maintained bone microstructure, decreased TRAP and RANKL expression, and decreased bone metastatic markers, relative to unloaded controls.[7,8,10,12] However, animal models may not capture the interactions of often rely on immunocompromised animals, which may not reflect human responses to disease progression. The goal of this study was to quantify the effects of mechanical stimulation on cancer cell engraftment and bone physiology. We 1) developed a novel model of cancer metastasis to human trabecular bone explants, and 2) quantified epithelial tissue and bone remodeling activity in loaded and unloaded samples.

METHODS: Femoral heads were obtained from total hip arthroplasty surgeries under informed consent and IRB approval from 7 patients (2 male, 5 female, average age 65 ± 10). Three to six cylindrical trabecular bone explants approximately 10 mm tall, 9 mm diameter were obtained from each femoral head. A 3 mm diameter hole was drilled through the center of each bone explant for implantation of gel encapsulated cancer cells, which were implanted after 72 h. TdTomato expressing MDA-MB-231 cells were cultured and formed into spheroids using the hanging drop method. Spheroids were suspended in 25 mg/mL fibrin solution which gelled in the explant cores for 30 min. Twelve explants (N=4 from 3 patients) cultured with gels embedded with cancer cells were cultured in a bioreactor system for 4 w, with 2 explants from each patient subjected to mechanical loading of 1.7 MPa at 5 Hz for 5 min twice a day, 5 times per w. After culture, bones were demineralized and stained with H&E. Tumor area from one section in each bone was measured using ImageJ. To visualize cancer cell invasion bone explants from 6 patients cultured with gels embedded from cancer cells were cultured in 12 well plates for 6 d. Confocal imaging of cancer cells (red) and bone (blue) were obtained every 15 min for the first 24 h of culture. A final confocal image was taken on day 6 of culture.

RESULTS: Epithelial regions indicating tumor formation were found in the bone explants (Fig. 1A,B). The epithelial tissue area was marginally greater in unloaded explants compared to loaded explants (p=0.13) (Fig. 1C). We detected more active bone resorption areas in unloaded bone explants (p=0.06) (Fig. 1G). Time lapse confocal microscopy at 0-24 h showed cancer cells escaping from spheroids, migrating towards bone structures, and attaching to the bone surface (Fig. 1D,E). Proliferation of cancer cells in the bone microenvironment was evident after 6 days of culture (Fig, 1F).

DISCUSSION: Bone is subjected to regular mechanical loading that affects the normal physiology of the bone and marrow cells. In order to understand the interactions of mechanical stimuli and cancer metastasis, we created a novel model of metastasis to human bone. We found that MDA-MB-231 cells initially attach to and migrate on bone surfaces, followed by formation of epithelial like regions within the trabecular pore space. Mechanical loading decreased the area of epithelial tissue and the occurrence of bone remodeling regions within trabecular struts. Our results are consistent with previous reports in mouse models.[8] The use of human bone increases the applicability of our model to drug screening and for hormone positive cancers that do not reliably metastasize to bone in mouse models.

SIGNIFICANCE/CLINICAL RELEVANCE: Cancer metastasis to bone is associated with altered bone physiology and calcium regulation which increases the risk of fracture, altered hematopoiesis, and death.

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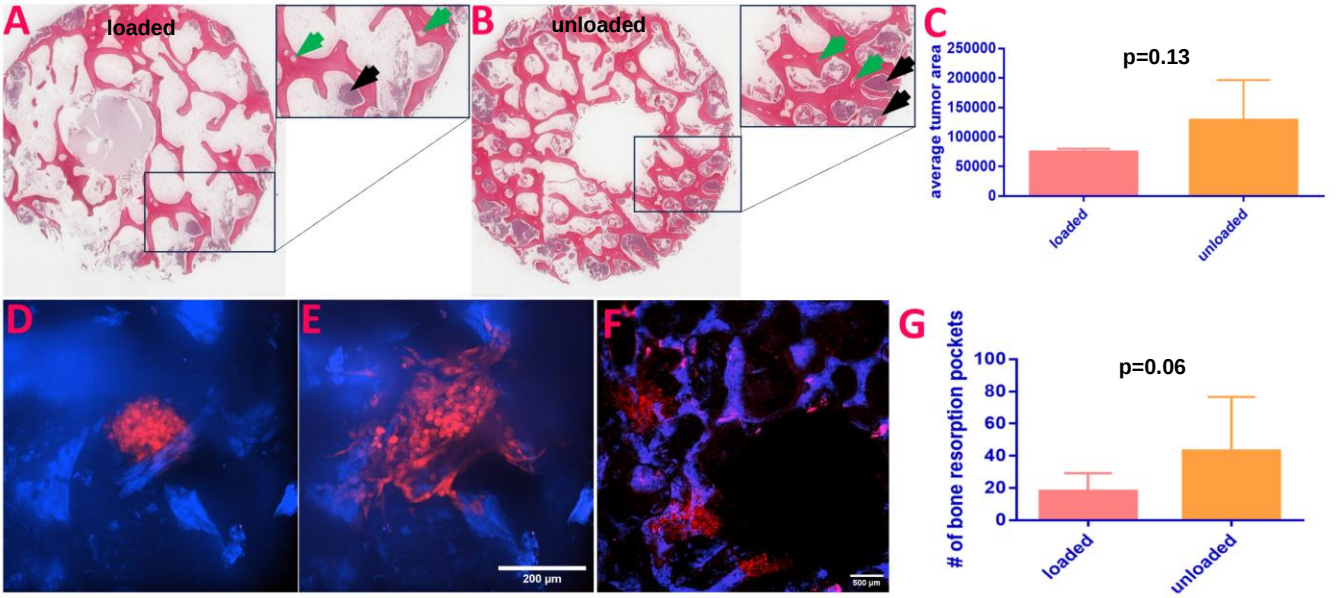

Figure 1: Representative H&E images of A) loaded and B) unloaded bone explants from the same patient after 4 weeks of bioreactor culture. Black arrows indicate tumor location, green arrows indicate active BMUs in the bone. C) Area of cancer cell coverage in histological slides after 4 weeks. Representative confocal time lapse images of MDA-MB-231 spheroids in bone at D) 0 hours and E) 24 hours and at F) 6 d. G) Average number of BMUs in histological slices after 4 weeks.