Differential osteosarcoma growth in loose areolar, musculofascial, and bone compartments: Importance of microenviromental stiffness

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Introduction: Surgical margins are a paramount concern in resection treatment of osteosarcomas and connective tissue sarcomas. The aggressive potential of osteosarcomas are often seen to be affected by the anatomic compartment of the tumor mass. Musculoskeletal oncologists use various metrics such as marginal tissue characteristics (positive/loose areolar/muscle/fascia/bone margins), marginal/wide/radial margin type, and intra-/extra-compartmental anatomic location to grade diseases as R0 (clear)/R1 (positive microscopic tumor cells)/R2 (positive gross tumors). Tumors that infiltrate certain compartments can have exceptionally poor outcomes, such as pelvic osteosarcoma which has disease-free survival rates as low as 19%. Current literature posits that pelvic osteosarcoma outcomes are due to extracompartmental location, insidious growth, delayed diagnosis, and technical difficulty of achieving negative surgical margins. We hypothesize that mechanical stiffness of tumor microenvironments is inversely related to growth of osteosarcomas of the same cell lineage.

Materials and Methods: We conducted in vitro and in vivo experiments simulating osteosarcoma growth in different microenvironments with varying biomechanical stiffness. We cultured the well-established 143B human osteosarcoma cells in 3-dimensional gels at 50Pa, 350Pa, and 1500Pa stiffness and measured tumor proliferation and cell density. Next, we implanted GFP-luciferase-143B human osteosarcoma cells inside the tibia (bony compartment), at the bone muscle interface (Enneking Stage IIB high-grade osteosarcoma abutting the muscle/fascia), and in a pelvic location (extracompartmental retropelvic) (N=6/group). We monitored clinical wellness, tumor size, and tumor cell proliferation, i.e. viable cancer burden, by in vivo bioluminescence imaging for 6 weeks after implantation. After 6 weeks, the mice were harvested to collect blood serum for RT-PCR and to process the tumor masses for IHC.

Comment [SL1]:

Results: Osteosarcoma cells grew more aggressively and rapidly in gels with lower stiffness (Figure 1, $p<0.05$). Likewise, osteosarcoma cells grew into a larger tumor mass faster in the retropelvic compartment than in the compartments surrounded by muscle/fascia or bone (Figure 2, $p<0.05$). The blood serum will be used for RT-PCR to identify circulating tumor cells – a metric that will be useful in establishing the metastatic potential of the 3 different compartments. IHC for cell proliferation markers and functional activity markers such as KI-67 and MMP9/13 will be performed on the tumor mass.

Discussion: Our results suggest that mechanical stiffness of cancer microenvironments changes the tumor biology with respect to cancer expansion. Bone marrow has rich blood supply and nutrition and yet did not provide the environments for the fastest growth despite aggressive osteolysis by the cancer in the tibial compartment. The relatively less stiff retropelvic compartment proved to be a fertile ground for rapid and invasive growth. Further studies defining specific molecular mediators responsible for aggressive expansion, tissue destruction, proliferation, and angiogenesis will lead to development of new adjuvant therapies.

Conclusions: Surgical margins do matter. R1 or R2 margins outside the bone will most likely cause aggressive recurrence especially in the pelvis, supporting current clinical observations.

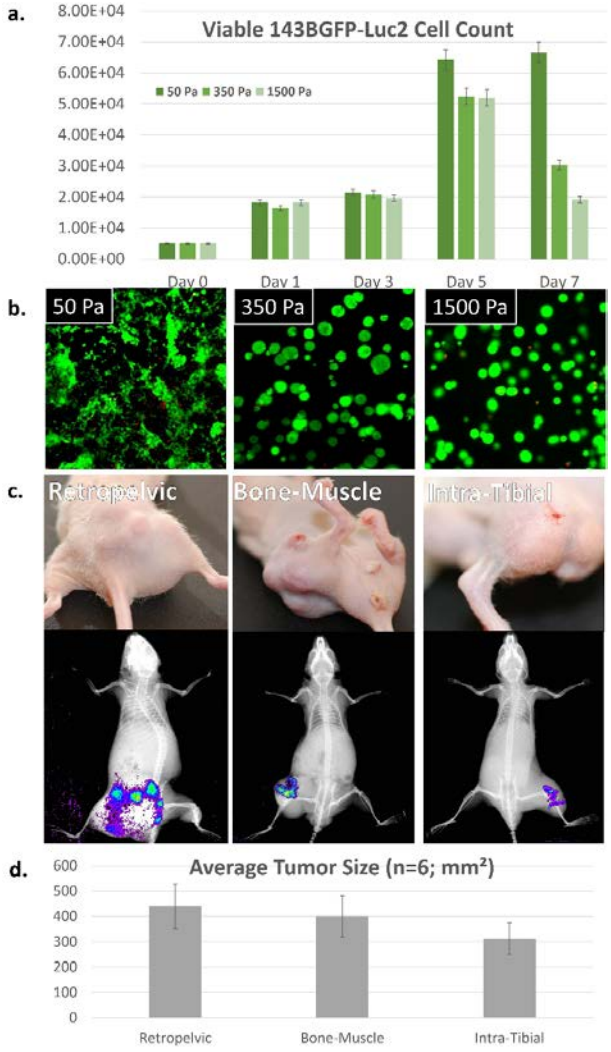

Figure 1