Defining the impact of TGFβ-targeted therapy on bone metastatic prostate cancer: an integrated biological/mathematical modeling approach

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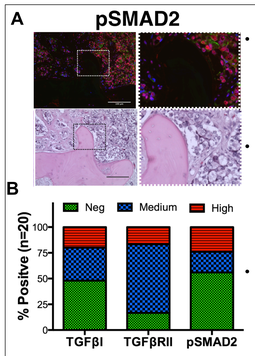
1. Introduction



- Bone metastatic prostate cancer is incurable. Tumor progression in bone is driven by a milieu of cellular and molecular interactions, occurring both simultaneously and in parallel, that produce regions of abnormal osteolysis and osteogenesis.
- TGFβ signaling is highly active in human prostate to bone metastases, yet, because of its noted pleiotropic effects it is difficult to fully define its effects on the tumor-bone microenvironment. Thus, it is unknown if therapeutic inhibition would be efficacious.
- Integration of computational modeling and biological systems allows for interrogation of can facilitate optimization of potential therapies and the function of complex molecules, such as TGFβ.

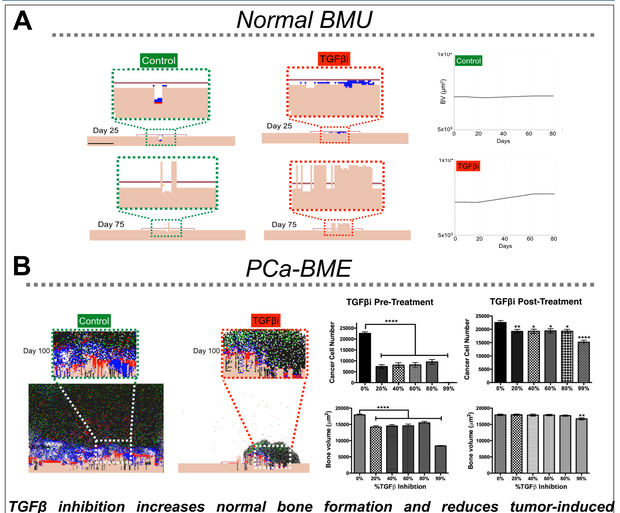
TGFβ signaling in patient prostate to bone metastases. **A,** Immunohistochemistry images of phospho-SMAD2 (top, red) in bone stromal cells and tumor (green); **B,** Quantitation of TGFβ receptor and pSmad2 expression.

2. Hypothesis & Methods

Hypothesis: Using a discrete hybrid cellular automata model (HCA) combined with in vivo models, we can predict the efficacy of therapeutically targeting TGFβ in prostate cancer to bone metastases.

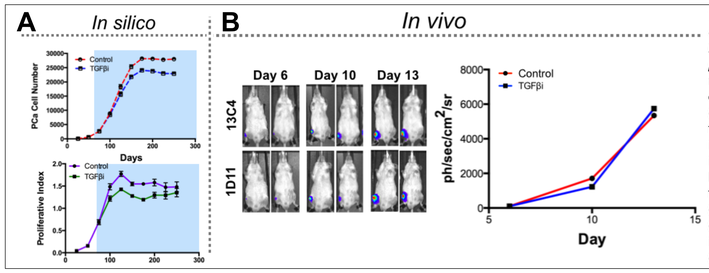
To develop our HCA model, we parameterized partial differential equations with empirical and published data. *In silico* simulations of 250 days (n=27) were performed for five different levels of TGFβ inhibition (0-100%), applied either pre- or post- metastatic seeding and were tested in vivo with an osteogenic and TGFβ responsive model of bone metastatic prostate cancer (PAIII) using a TGFβ neutralizing antibody, 1D11.

3. Impact of in silico TGFβ inhibition on normal bone remodeling and tumor-induced bone formation

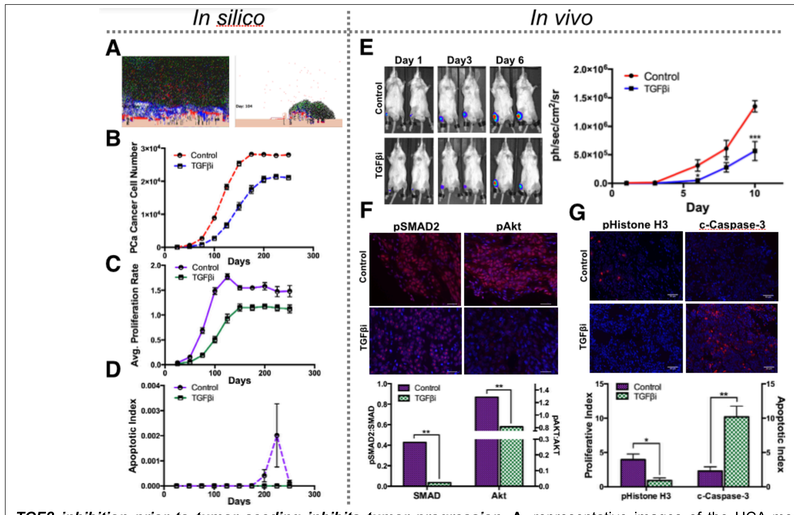


TGFβ inhibition increases normal bone formation and reduces tumor-induced osteogenesis. **A,** normal bone formation remodeling shown in snapshots (left) of HCA at Days 25 and 75, line graphs represent bone formation;**B,**TGFβ inhibition prior to tumor seeding (pre-treatment) inhibits tumor growth and bone formation. Left, images of pre-treatment HCA model at Day 100 of 250 days; right, graphs represent tumor growth and bone formation at different TGFβ inhibitor doses, pre-and post-treatment.

4. *In vivo* TGFβ inhibition testing of *in silico* predictions of tumor growth

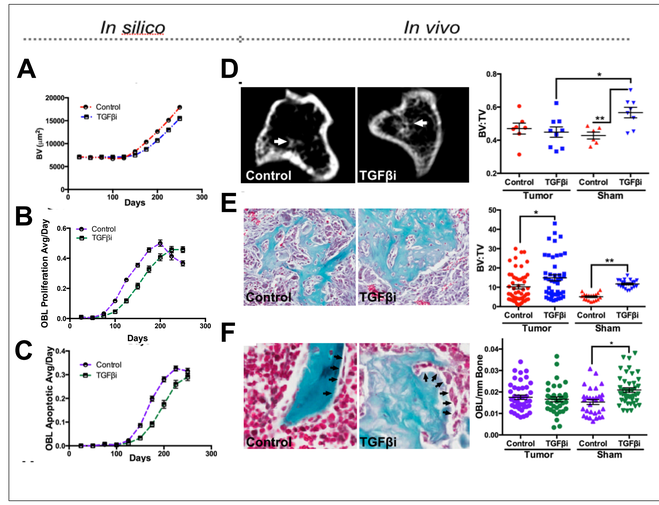


TGFβ inhibition of an established tumor has little impact on tumor progression. **A,** Graphs (left) represent *in silico* predictions of TGFβ inhibition applied (blue shading) as a post treatment after tumor seeding in bone; **B,** Images represent intratibial bioluminescence of luciferase-expressing PAIII treated with either 1D11 (TGFβ antibody) or 13C4 (control), at Day 6 after tumor inoculation, and quantitation (right).



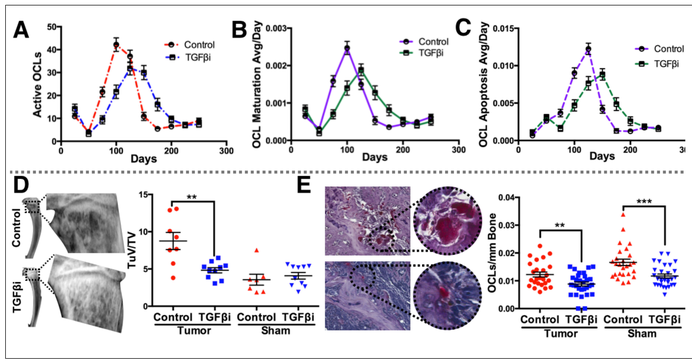
TGFβ inhibition prior to tumor seeding inhibits tumor progression. **A,** representative images of the HCA model, PCa-BME (prostate cancer-bone microenvironment), control (left) and with TGFβ inhibitor(right); **B-D**, Graphs show *in silico* tumor burden, proliferation and apoptotic indices; **E,** representative *in vivo* bioluminescence (left) and quantitation (right) of SCID Beige mice inoculated with PAIII one day after 1D11 treatment; **F-G,** immunohistochemistry of Akt and Smad signaling (left), phosphohistone H3, and cleaved caspase 3.

5. Impact of TGFβ inhibition on tumor-induced osteogenesis



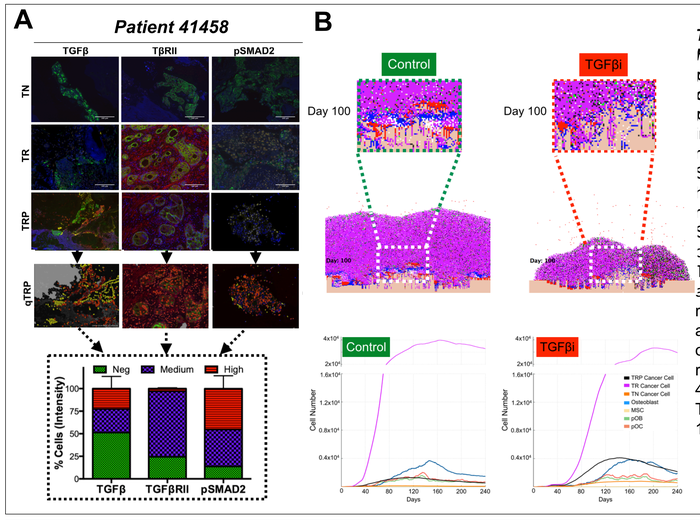
TGFβ inhibition does not exacerbate tumor-induced osteogenesis. **A-C,** Graphs illustrate impact of TGFβ inhibition on bone volume and osteoblast proliferation and cell death;**D,** Representative images (left) and quantitation (right) of microcomputed tomography (μCT) analysis; **E-F,** representative images and quantitation of *in vivo* bone volume to tissue volume (E) and total osteoblasts per mm bone(F) using Trichrome histological stain.

6. Impact of TGFβ inhibition on bone osteolysis *in vivo* and *in silico*



TGFβ inhibition reduces osteolysis. **A-C,** representative graphs of total number of active osteoclasts(OCL), OCL maturation/fusion, and OCL apoptosis; **D,** *in vivo* x-ray images of tumor-bearing tibia and quantitation of lesions per 2mm bone (right); **E,** images of TRAcP-positive osteoclasts, graph represents total number of TRAcP-positive OCLs per mm of bone.

7. Impact of TGFβ inhibition of Heterogenous Tumor using mCRPC patient data



TGFβ inhibition of heterogenous tumor population identifies outgrowth of resistant population. **A,** histological images of TGFβ ligand, receptor (TGFβRII), and Smad signaling (pSMAD2)in human metastatic castration resistant prostate cancer. Signal intensity was quantified using Definions Tissue Studio (lower panel) and used for HCA model; **B,** representative images (top) and quantitation (lower panel) of heterogeneous TGFβ responsiveness in Patient 41458 with pre-treatment TGFβ inhibitor (right) at Day 100 of 250 day simulation.

8. Conclusions and Future Directions

- Integrated findings from our HCA and *in vivo* model demonstrate that TGB therapy would be beneficial for targeting bone metastatic prostate cancer when applied at early stages in tumor progression.
- Using the HCA computational model, we were able to examine changes in the tumor microenvironment at distinct time points and to identify the optimal therapeutic window for preventing tumor growth, without exacerbating tumor-induced osteogenesis.
- In combination with patient data, our HCA model can be utilized to identify emergence of tumor populations resistant to targeted therapies.

Future Directions

- Our HCA computational model is broadly applicable to studying the tumor microenvironment of other cancer types. In the near future, the model will be used as a predictive tool for optimizing putative therapies of bone metastatic prostate cancer .

9. Support

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