Targeting The BMP Pathway In Prostate Cancer Induced Bone Disease

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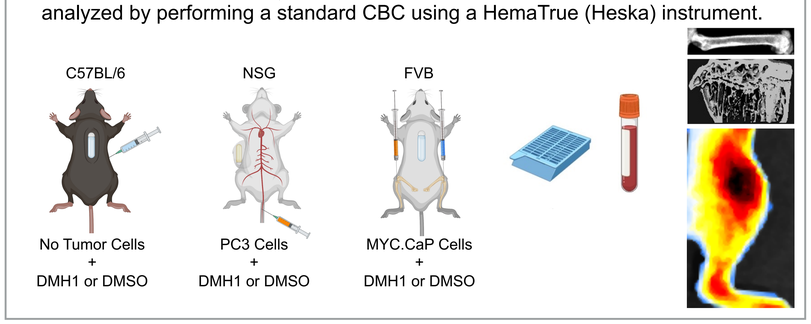
Abstract

From the 33,000 men in the U.S. who die from prostate cancer each year, the majority of these patients exhibit metastatic disease with bone being the most common site of metastasis. Prostate cancer bone metastases are commonly blastic, exhibiting new growth of unhealthy sclerotic bone, which can cause painful skeletal related events. Patient's current care entails androgen deprivation therapy, anti-resorptive agents, radiation and chemotherapy to help control the spread of the cancer but little intervention is available to treat blastic bone disease. The transforming growth factor beta (TGFβ) and bone morphogenetic protein (BMP) pathways are known to regulate bone growth and resorption of destructive lytic bone lesions, yet the role of TGFβ/BMP signaling in prostate cancer blastic bone lesions are not fully understood. We hypothesized that in order to target the BMP/TGFβ pathway a useful biomarker of bone lytic or blastic pathology would have a superior response.

Methods

- We used clinical archived FFPE decalcified bone samples to detect differences in lytic or blastic pathologies using IHC staining.
- BMPs exhibit distinct effects on bone homeostasis, so to examine the effect of BMP inhibition on healthy bone, we treated mice with the BMP receptor small molecule antagonist dorsomorphin homolog 1 (DMH1)
- We next sought to use the BMP inhibitor DMH1 to treat bone metastasis seeded by a caudal artery injection of the lytic human prostate cell line PC3 in immunodeficient mice. (Natagawa,K. *et al.* A Reliable murine model of bone metastasis by injecting cancer cells through caudal arteries. *Nat Commun* 9, 2981 (2018)
- We next proceeded to test BMP inhibition in an injury model of bone metastasis via intratibial injection of the MYC.CaP mouse prostate cell line into FVB syngeneic mice.
- Data collection was performed using the following imaging modalities: DXA (Faxitron) was used for multiple data points to measure BMC and BMD during the studies, μCT (1276 SkyScan) was used to quantitate trabecular and cortical bone measurements (data not shown) and total radiance detection using RediJect2-DeoxyGlucosone (DG) 750 (Perkin Elmer) using the IVIS Spectrum (Perkin Elmer).
- Peripheral blood was analyzed. Venipuncture via submandibular blood collection analyzed by performing a standard CBC using a HemaTrue (Heska) instrument.



Human Metastatic Prostate Cancer in Bone

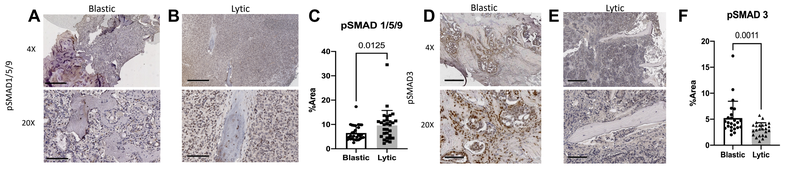


Figure 1. Human Metastatic Prostate Cancer in Bone Express Distinct TGFβ and BMP Signaling. Canonical BMP signaling can be detected by the nuclear translocation of BMP specific SMAD1/5/9 whereas canonical TGFβ signaling can be detected by nuclear translocation of SMAD3, which are both active when phosphorylated. **(A)** Blastic bone pathology from human patients with metastatic prostate cancer at low power (above) and high power (below) stained for pSMAD1/5/9 by IHC. **(B)** Lytic bone pathology from human patients with metastatic prostate cancer at low power (above) and high power (below) stained for pSMAD1/5/9 by IHC. **(C)** Quantification of DAB positive IHC staining by total percent area is graphed for at least five distinct fields of view for no less than 6 patients per group and analyzed for significance by Mann-Whitney t-test and error bars indicate SD. **(D-F)** Blastic and lytic samples IHC stained for pSMAD3 and compared for expression between blastic and lytic pathology. Scale bars for 4X and 20X are 200μm and 50μm respectively

Evaluation of Bone With DMH1 Treatment

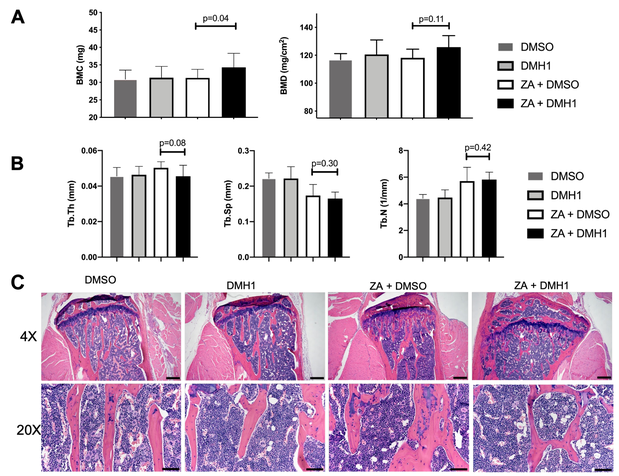


Figure 2. BMP Receptor Antagonist DMH1 Does Not Impair Bone. Twenty male 8-week-old C57Bl6/J mice were placed into four groups (n=5 each) where they received a 6-week osmotic pump of DMSO or DMH1 alone or in addition to Zoledronic acid. 6 and 12 weeks after initial treatment with the osmotic pumps, the animals were analyzed by DXA Faxitron X-ray. At week six, osmotic pumps with DMH1 or DMSO expired, the animals received an additional injection of zoledronic acid and were then monitored for another 6 weeks. **(A)** BMC and BMD analysis of DXA data from week 6. **(B)** Analysis of μCT values of the femoral trabecular bone at week 12. **(C)** Representative H&E images of femur bone with emphasis in trabecular area. Scale bars for 4X, 10X and 20X are 200μm, 100μm and 50μm respectively. Statistical significance was determined using an unpaired one-tailed t test (Mann-Whitney) error bars indicate SD.

Human Prostate Cancer Cell Line With Treatment

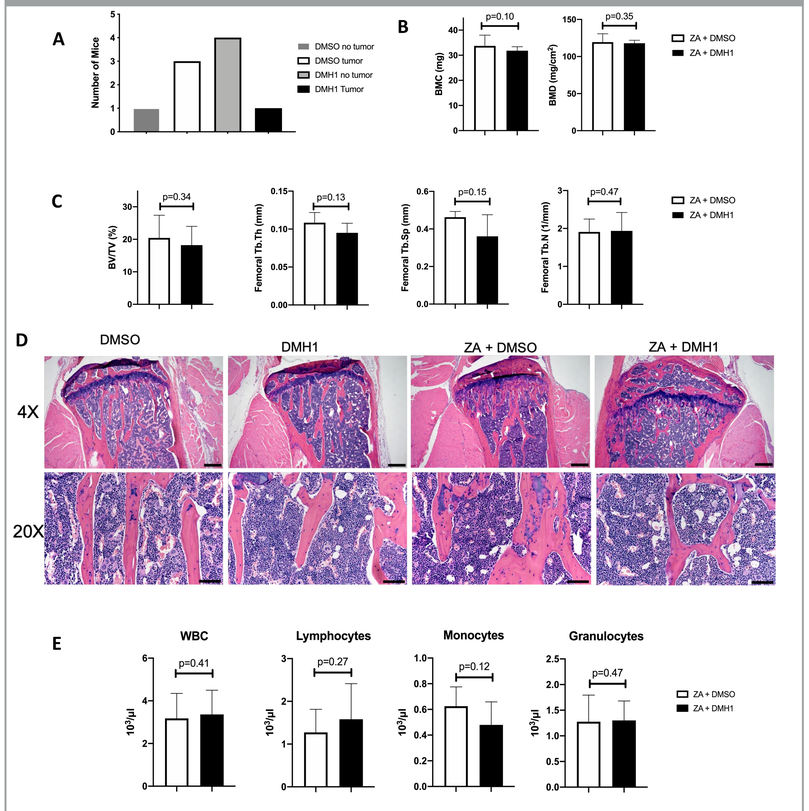


Figure 3. Caudal Artery Injected Human PC3 Osteolytic Prostate Cancer Cells Are Restricted for Engraftment with BMP Inhibition. Nine male 8-week-old NSG mice received 50,000 PC3 cells via caudal artery injection. All mice received an IP injection of zoledronic acid, and subcutaneous 4-week osmotic pump implant containing DMSO (n=4) or DMH1 (n=5). **(A)** Analysis of tumor incidence, with the DMSO treated group (n=4) having three mice with metastatic tumors in bone, while the DMH1 treatment (n=5) group having one mouse with metastatic tumor in bone. **(B)** BMC and BMD measurements from femur of DXA imaging. **(C)** μCT data analyzed from femur trabecular bone (DMSO n=2, DMH1 n=5 analyzed for quantification). **(D)** H&E representative images of tumor invasion located in the femurs. **(E)** Complete Blood Count (CBC) from peripheral blood at time of euthanasia. Scale bars for 4X, 10X and 20X are 200μm, 100μm and 50μm respectively. Statistical significance was determined using an unpaired one-tailed t test (Mann-Whitney) error bars indicate SD.

Injury model of Syngeneic Prostate Cancer Cells

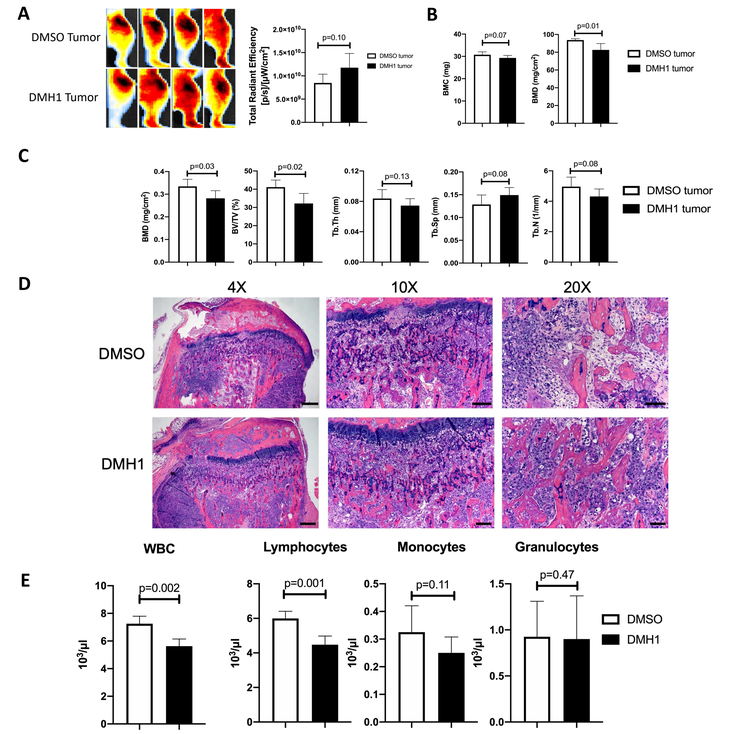


Figure 4. Tibial Injected Mouse MYC-CaP Syngeneic Prostate Cancer Cells Are Unchanged for Engraftment with BMP inhibition. Eight male 8-week-old FVBn mice received 100,000 MYC-CaP cells via tibial injections in the left leg, with 10μl PBS being injected into the right tibia as a control. Animals were placed into treatment groups of DMSO (n=4) or DMH1 (n=4) with 4-week osmotic pumps and all mice were given an IP injection of zoledronic acid. **(A)** IVIS fluorescence detection using 750 2-DG mice were intravenously injected 5 hours before data acquisition at the expiration of the osmotic pump (4 weeks). Image shows ROIs of the tumor bearing left tibia. The graph indicates total radiant efficiency of the ROI. **(B)** Faxitron BMC and BMD measurements derived from selecting an ROI encompassing the whole tibia. **(C)** μCT bone health quantitation data from the tibia. **(D)** Representative H&E histology slides. **(E)** Complete Blood Count (CBC) from peripheral blood at time of euthanasia. Scale bars for 4X, 10X and 20X are 200μm, 100μm and 50μm respectively. Statistical significance was determined using an unpaired one-tailed t test (Mann-Whitney) error bars indicate SD.

Conclusions

It is important to determine whether patients have a lytic or blastic phenotype of bone disease, as the matrix physiology could dictate disease progression. Patients' willingness to provide direct samples (biopsies) could potentially improve their treatment. However, the discovery of a biomarker responsible for this cascade will be the most useful. Treatment of DMH1 did not have a prolonged effect or change the outcome of robust tumor burden, but may offer protection from metastasis to the bone. Nevertheless, we are optimistic in finding other BMP pathway inhibitors to treat the sclerotic phenotype. An impact can be made by reducing the poor quality of destructive or sclerotic bone in prostate cancers.

Future Directions

Using our current animal models and organoid model, we would like to enhance the model translationally:

- Add chemotherapies (Docetaxel) for treatment.
- Add ADT (Abiraterone/Enzalutamide)
- Add targeted radiation therapy (RT) with ablative/fractionated doses.
- Increased resolution to detect cancer cells which extravasate to the bone (i.e. IVIS probes) for TIBD.
- Target blastic models with BMP/TGFβ inhibitors.

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