Multimodality uCT/optical imaging reveals acute sorafenib-driven increases in bone loss and inflammation in a model of breast cancer growth in bone

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Abstract

Tumor metastasis to bone occurs with high incidence in advanced breast cancer patients, frequently leading to skeletal complications such as osteolytic lesions and fractures due to the activation of bone degrading osteoclasts. Effective treatments often include combinations of chemotherapy or antibody therapy, combined with bisphosphonate treatment to ameliorate bone damage. To investigate tumor-induced bone loss, and the impact of treatment, we implanted luciferase-expressing 4T1 breast adenocarcinoma cells into the right knee joints of syngeneic nu/nu mice. We used non-invasive bioluminescence imaging (BLI), near infrared (NIR) fluorescence imaging (FLI), and μCT measurement of bone changes to assess tumor burden, biological changes, and bone changes, respectively. Sorafenib, a clinically approved multi-kinase inhibitor, was used as a treatment (PO 100 mg/kg, day 4 & 7 post-tumor implantation) to inhibit tumor metabolic activity and angiogenesis. Zoledronic acid, a bisphosphonate drug, was used for uCT imaging reveals acute bone resorption associated with tumor growth. Tumor burden was assessed on day 8 by BLI tomography on the IVIS® SpectrumCT, revealing that bisphosphonate treatment effectively decreased tumor burden by 60%, whereas Sorafenib decreased tumors by 75%. Fluorescence imaging using imaging probes specific for cathepsin K and cathepsin B suggested that despite reduction of tumor size by treatments, Cat B total signal stayed the same. Correction for tumor size revealed local 2- and 3-fold enhancement in Cat K and Cat B tumor signal, respectively. For μCT analysis on the Quantum GX, we developed novel semi-automated image processing approaches for quantitative morphometric analysis, with auto-segmentation of femoral and tibial epiphysis & metaphysis. This analysis revealed changes in bone volume, associated with both untreated tumor growth and with treatments. Untreated mice showed a 75% decrease in femoral trabecular bone volume as compared to the contralateral knee, Zoledronate treatment decreased this trabecular bone volume loss to ~4%, however Sorafenib treatment, despite decreased tumor burden, showed a surprising lack of protective effect on trabecular volume and a 2-fold decrease in the trabecular bone volume fraction as compared to normal, untreated, and zoledronate-treated mice, as a result of tumor induced trabecular bone damage, which we attribute to increased focal inflammation. These results agree well with observations in the literature and further suggest that multimodality imaging quantifies both positive and negative structural and biological changes associated with treatment of bone metastases.

1 Imaging Systems and Probes

<table>
<thead>
<tr>
<th>Imaging Systems</th>
<th>Description</th>
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<tr>
<td>Quantum GX microCT Imaging System</td>
<td>High resolution, high speed, microCT system for preclinical imaging</td>
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<tr>
<td>IVIS SpectrumCT in Vivo Imaging System (Edinburgh)</td>
<td>Integrated optical bioluminescence &amp; fluorescence and microCT practical imaging system</td>
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<th>Reagents</th>
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<tr>
<td>Cat K 680 FAST™</td>
<td>Cat K-activatable NIR FLI probe, expressed in osteocytes and tumor associated macrophages</td>
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<tr>
<td>Cat B 750 FAST™</td>
<td>Cat B-activatable NIR probe expressed in inflammatory cells and tumor cells</td>
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<tr>
<td>Zoledronate (Sigma-Aldrich, St. Louis, MO)</td>
<td>Bisphosphonate drug used to treat bone diseases</td>
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<tr>
<td>Sorafenib (Siga-Aldrich, St. Louis, MO)</td>
<td>Tyrosine kinase inhibitor, used to treat multiple myeloma patients in cancer progression</td>
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Practical imaging systems, probes, and treatments used to assess tumor growth, inflammation, and bone changes in a mouse model of tumor induced osteolysis.

2 4T1 Knee Tumor Implantation Model

Mouse Breast Cancer Bone Growth Model

Six to eight week-old female NU/NU mice were purchased from Charles River Laboratories (Wilmington, MA) and maintained on low-fluorescence mouse chow (Envigo (N.V.G., Madison, WI) vial). To induce cancer to the knee, NU/NU mice were injected into the right knee joint with 4T1-Red-Fluc cells (Harvard, CA) or NU/NU mice were injected into the left primary mammary fat pad with 4T1-Red-Fluc cells at a density of 6x10⁶. In addition NU/NU mice were injected into the left knee joint with 4T1-Red-Fluc cells at a density of 6x10⁶. Both sites of injection yielded tumors within ten days.

3 IVIS SpectrumCT 4T1 Knee Tumor BLI

A. 3D Bioluminescence Knee Tomography (DLIT) with μCT

B. 2D Bioluminescing Imaging (BLI)

Mice (n=6 per group) were either treated with Zoledronate (Sigma-Aldrich, St Louis, MO) or Sorafenib (LC Laboratories, Woburn, MA). A control group (n=4) was added for background comparison. Mice received Zoledronate (PO 100 μg/kg, day 0, 4 & 7) and Sorafenib (PO 150 mg/kg, days 0 & 7) treatment. A. 3D BLI images on the IVIS SpectrumCT (10 min post-bacterial injection IP) showing knee tumors and co-registered knee joint bone. B. 2D BLI images showing both knee and mammary fat pads tumor burden with quantification.

4 Treatments decrease tumor burden but increase localized cathepsin B and cathepsin K activity

A. IVIS SpectrumCT fluorescence imaging

B. Knee tumor fluorescence quantification

C. Mammary fat pad Tumor fluorescence quantification

The same representative mice from Figure 3 were injected with Cat K 680 FAST and Cat B 750 FAST on day 7 for imaging 24h later. A. 2D FLI images of mice on the IVIS SpectrumCT showing knee tumor cathepsin B and K activity. B. Knee tumor fluorescence quantification showing total (left panel) and BLI normalized signal (right panel) to control for tumor size. C. Mammary fat pad tumor fluorescence quantification for both total (left panel) and BLI normalized signal (right panel) to control for tumor size. Data is represented as biological activity intensity ± standard error. Symbols indicate statistical significance (p<0.05; *p<0.01).

5 AccuCT™ Software - Automatic Bone Segmentation

We established novel mathematical algorithms and software (AccuCT, PerkinElmer) for the purpose of generating semi-automated bone segmentation and analysis, avoiding labor intensive current analyses and subjectivity by defining segment boundaries. The diagram illustrates the bone detection/segmentation workflow.

6 Bone segmentation identifies drug-associated protection and exacerbation of tumor induced bone loss

A. Knee joint bone images

B. Bone metric quantification

Standard ASBMR bone readouts of trabecular and cortical bone changes were assessed for knee regions of each animal in each group using AccuCT Software. Segmentation and ASBMR analysis rendering images. B. Quantification of femur and tibia results. Black bars represent 3 untreated control contralateral knees. Data is represented as mean ± standard error, and symbols indicate statistical significance (p<0.05; *p<0.01).

7 References

1) Aleman JO et al. Effects of tyrosine kinase inhibition on bone metabolism: untargeted consequences of targeted therapies. Endocrine-Related Cancer (2014) 21, R247-R259