Localized Tumor Growth in Bone: Quantification of Tumor Burden, Bone Destruction and Weight Bearing
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Abstract
Metastatic bone disease is common in patients with multiple myeloma, breast, prostate, and lung cancer, and is a frequent cause of morbidity in advanced cancer patients. Metastatic bone tumors both destroy bone and cause severe bone remodeling, leading to structural weakening and bone fractures in a large percentage of patients, and often resulting in severe bone pain. These studies examined tumor growth, bone erosion, and pain in a nude rat model. A surgical defect was created in the tibia, exposing the marrow cavity. Luciferase-expressing MDA-MB-231 mammary tumor cells were injected into the exposed bone cavity. Rats inoculated with tumor cells were treated with either doxorubicin (3 mg/kg/wk, iv) or risedronate (80 µg/kg/day, ip). Animals were monitored weekly for tumor burden by optical imaging and for bone erosion by CT. In addition to clinical observations, weight bearing was measured by placing the rats in an incapacitance tester with each hind paw on a force transducer. Compared to controls, rats treated with doxorubicin demonstrated a significant decrease in luminescence, indicating tumor growth inhibition (bone protection). Decreased luminescence correlated with sparing of the bone as measured by CT. In contrast, the bisphosphonate risedronate had limited effect on tumor growth, but preserved bone integrity. In both treatments, protection of bone was associated with normalization of weight bearing. This finding supports the premise that pain in metastatic bone cancer is due to bone loss (destruction) and remodeling. Weight-bearing data correlated directly with clinical observations. This model, leveraging multiple imaging modalities, enables longitudinal and quantitative assessment of tumor growth; bone loss, remodeling and sparing; and weight bearing in test animals and therefore provides a sensitive and robust screening tool to evaluate compounds for anti-tumor, bone-sparing and pain amelioration effects.

Study Design

Male nude rats were inoculated with Luciferase-expressing MDA-MB-231 (human breast cancer cells). After 5 days, rats were screened for presence and size of tumors, and randomized into treatment groups.

Starting on Day 5, rats (n=10/treatment group) were given vehicle (saline), Actonel® (risedronate; 80 µg/kg/day, ip) or doxorubicin (3 mg/kg/wk, iv).

Optical imaging to assess tumor growth and volume were assessed in the same rat on multiple time points.

Weight bearing was assessed on multiple time points as an indirect measure of bone pain.

Bone volume and mineral content (bone erosion) were assessed at study termination using CT on dissected specimens.

Materials and Methods

Tumor Cell Inoculation Procedure

Rats were anesthetized using 3% isoflurane.
The surface of the tumour and tibia were exposed using a surgical skin incision and blunt tissue dissection.

An injection entry site was created using a 25-ga needle inserted through the mid-point of the medial surface of the tibia, just distal to the physes.

200,000 Luciferase expressing tumor cells (MDA-MB-231-Luc, human breast cancer) were injected into the right tibia using a 30-ga needle stub.

Osteonecrosis was applied to the injection site to assist with hemostasis and the surgical site was closed with surgical adhesive.

Xenograft IVIS Bioluminescence (Optical) Imaging

Rats were injected with Lucifiren Firefly probe (150 mg/kg in saline), ip.

10 min post-injection animals were anesthetized with 3% isoflurane.

Data was analyzed with Living Image® 4.0.

Weight Bearing

Downward force for each hind limb in each rat was measured using an incapacitance tester (Model 1029, Columbus Instruments, Columbus, OH).

After appropriate placement of the rat in the restrainer, the force on each transducer was recorded for each limb (4 samples/second) for 20 seconds.

To ensure that excessive movement would not impact analysis, set software criteria were used to set a “threshold” or “minimum to maximum range” for each hind limb.

GE Locus SPMicro-CT

Extracted tibias were imaged via the specimen CT.

Images were acquired using a 30-µ scan, 80 kV 80 uA, 400 views, 0.5 degree angle, 1700 msec exposure.

Images of interest were drawn from the proximal tibial growth plate to the junction of the tibia and fibula.

Bone mineral density (BMD) and bone mineral content (BMC) were calculated using MicroView® analysis software.

Results

Bone loss in MDA-MB-231-Luc tibia tumor bearing rats given vehicle, doxorubicin or Actonel® (n=10)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BMC (mg)</th>
<th>Volumetric Ratio</th>
</tr>
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<tbody>
<tr>
<td>Vehicle</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>105</td>
<td>0.95</td>
</tr>
<tr>
<td>Actonel®</td>
<td>110</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Figure 1. Bioluminescence and CT images from each treatment group at study termination.

Figure 2. Bone volume and bone mineral content.

Figure 3. Bone volume and bone mineral content.

Figure 4. Weight bearing in rats given vehicle, doxorubicin or Actonel. Note that rats given vehicle have an increase in L/R leg ratio, demonstrating that more weight is borne on the non-tumor bearing side.

Conclusions

Risedronate, a bisphosphonate, prevents bone breakdown and increases bone density.

Doxorubicin is a chemotherapeutic agent used to treat a variety of cancers.

Quality of life (pain mitigation) is an important consideration in treatment of metastatic bone cancer.

Bone loss and remodeling are considered to be important factors in pain.

Compared to controls, rats treated with doxorubicin demonstrated a significant decrease in luminescence, indicating tumor growth inhibition, that correlated with sparing of the bone as measured by CT.

Doxorubicin had limited effect on tumor growth, but preserved bone integrity.

Normalization of weight bearing was seen in rats given risedronate or doxorubicin, supporting the hypothesis that pain in metastatic bone cancer is due to bone loss (destruction) and remodeling. Weight-bearing data correlated directly with clinical observations (data not shown).

This model enables longitudinal and quantitative assessment of tumor growth; bone loss, remodeling and sparing; and weight bearing in test animals and therefore provides a sensitive and robust screening tool.
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