Quantitative image analysis method for measuring whole-body tumor burden in a mouse model of breast cancer bone metastasis

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Introduction
Non-invasive fluorescence imaging is a promising new tool with several advantages over such imaging methods as radiography and histomorphometry, which are commonly used in cancer animal models. Fluorescence imaging is fast and allows whole body imaging without the need of sacrificing the animals. Unlike radiography and histomorphometry, whole-body fluorescence imaging is also not limited to very small region of interest. However, the means of transforming the imaging output into descriptive and reliable quantitative data have been limited. The most commonly used output data, fluorescence emission area as an index of tumor burden does not, however, take the fluorescence signal intensity into account. This limits the use of fluorescence imaging as a sensitive and reliable tool in testing new therapies against cancer. Furthermore, tumor burden and osteolysis is conventionally measured only in histological sections of hind limbs and radiographs. Yet, the correlation between these two methods and fluorescence imaging has been poorly studied.

Aim of the study
Our goal was to validate a new image analysis method for preclinical, screening-type drug efficacy research using GFP-MDA-MB-231(SA) breast cancer cells in a mouse model of breast cancer bone metastasis (1,2).

Materials and methods
- Cell culture: MDA-MB-231(SA) breast cancer cells transfected with pTurboGFP-N vector (Evrogen JSC, Moscow, Russia) were cultured in 10% FCS/DMEM medium supplemented with 1% non-essential amino acids, penicillin/streptomycin.
- Breast cancer bone metastasis mouse model: Anesthetized (Ketamine/Xylazine), female nude mice were inoculated intracardially with GFP-MDA-MB-231(SA) breast cancer cells (10^7). Three study groups were included (n=12/group): 1) Vehicle, 2) Doxorubicin 2.5 mg/kg, 3) Doxorubicin 5 mg/kg (all groups administered i.p. once a week). The tumor burden was assessed at day 14 and at the end of the study (day 23) by measuring the total area and signal intensity emitted by GFP-MDA-MB-231(SA) cells using LT9-Macro Imaging Systems, (Lightools Research, Encinitas, CA, USA; exposure 3.3s, gain 4.85 and offset -440). Osteolytic lesion area was measured on day 14 and at the end of the study by radiography using Faxitron MX20-DC2 (Faxitron Corp., Wheeling, USA). At necropys, all macroscopic signs were recorded and tissue samples were collected for histology.
- Image analysis: Total lesion number and area in hind limbs per mouse as measured by radiography were quantitated from the images using Metamorph image analysis software (Molecular Devices, Downingtown, USA). Tumor burden in GFP images, and total tumor area per hind limb in decalcified and H&E stained sections of right hind limbs were determined using Metamorph.

Correlations

Table 1. The results from tumor burden analyses as measured by fluorescence correlate with histomorphometry and also with osteolytic lesion areas in vehicle-treated mice. p-values determined using Spearman rank order correlation.

<table>
<thead>
<tr>
<th>Tumor area (histology, total burden)</th>
<th>Correlation</th>
<th>p-value</th>
<th>Tumor burden (GFP, total burden)</th>
<th>Correlation</th>
<th>p-value</th>
<th>Tumor burden (GFP, vehicle burden)</th>
<th>Correlation</th>
<th>p-value</th>
<th>Osteolytic lesion area (radiography)</th>
<th>Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.827</td>
<td>&lt;0.001</td>
<td>Vehicle</td>
<td>0.718</td>
<td>0.011</td>
<td>Vehicle</td>
<td>0.718</td>
<td>0.011</td>
<td></td>
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<td></td>
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<tr>
<td>Doxorubicin 2.5 mg/kg</td>
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<td>0.006</td>
<td>Doxorubicin 2.5 mg/kg</td>
<td>0.508</td>
<td>0.045</td>
<td>Doxorubicin 2.5 mg/kg</td>
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<tr>
<td>Doxorubicin 5 mg/kg</td>
<td>0.636</td>
<td>0.0321</td>
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Figure 1. Doxorubicin reduces tumor-induced osteolysis in a breast cancer model of bone metastasis. Representative figures of H&E-stained hind limbs of vehicle (A), doxorubicin (2.5 mg/kg) -treated (B) and doxorubicin (5 mg/kg) -treated (C) groups at sacrifice. Osteolytic lesion area was measured on day 14 and at the end of the study by radiography using Faxitron MX20-DC2 (Faxitron Corp., Wheeling, USA). At necropsy, all macroscopic signs were recorded and tissue samples were collected for histology.

Figure 2. Doxorubicin reduces tumor growth in bone and visceral organs in a breast cancer model of bone metastasis. Representative figures of fluorescence imaging in pre-study (A), post-study (B) and day 23 (C) of GFP-MDA-MB-231(SA) cells in hind limbs in the vehicle (p<0.05), doxorubicin (2.5 mg/kg, p<0.05) and doxorubicin (5 mg/kg, p<0.05) treated groups (versus vehicle) *equals p-value <0.05 (ANOVA followed by Dunn’s test).

Figure 3. Correlation between IADOS and osteolytic lesion area as measured by radiography in hind limbs of vehicle and doxorubicin 2.5 mg/kg treated mice. p-values determined using Spearman rank order correlation.

Discussion
These results demonstrate that doxorubicin effectively inhibits both cancer osteolysis and tumor burden in a mouse model of breast cancer bone metastasis. Our data suggests that GFP imaging is a fast and reliable tool that can be used as a quantitative method for screening-type assessment of whole-body tumor burden in a mouse model of breast cancer bone metastasis. Furthermore, this method can potentially replace the use of laborious and time-consuming histology for assessing tumor burden.

Conclusions
- GFP-MDA-MB-231(SA) cells retained similar tumor growth profile and metastatic characteristics as parental MDA-MB-231(SA) cells.
- The development of osteolytic lesions and tumor burden as measured by histomorphometry was inhibited by doxorubicin at 5mg/kg (Figures 1 and 3, respectively).
- Tumor burden as measured by fluorescence imaging was inhibited by doxorubicin at 2.5mg/kg and 5mg/kg (Figure 2).
- Tumor burden correlated with radiography as well as histological analysis of tumor burden at the sites where histological analyses were performed (Table 1).

Future studies
- Autofluorescence remains one of the key problems of fluorescence imaging and more validation is required to optimize the image acquisition procedures and specimen preparation.
- Future validation is needed to optimize the procedure of multi-aspect imaging to include the complete emission signal in the data output and fully utilize the newly created tumor burden index.

References