Effects of Mesenchymal Stromal Cells on Pulmonary Metastasis After Primary Tumor Removal in a Murine Model of Osteosarcoma

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Introduction: Mesenchymal stromal cells (MSC), which have a propensity to “home” to sites of injury or inflammation, aid native tissue in repair and regrowth. MSCs have been shown to improve bone integration and healing in several preclinical studies and have great potential for therapeutic use in limb salvage following massive bone loss due to trauma or tumor resection (1-3). However, MSCs have also been shown to promote primary and pulmonary metastatic tumor growth when injected in the presence of gross tumor or when co-injected with tumor cells in rodent models (4, 5). While these results raise concerns about the safety of using MSCs in cancer patients, MSCs are unlikely to be utilized in a clinical setting when gross tumor is present. Osteosarcoma (OSA) is the most common primary bone sarcoma. Surgical removal of the primary tumor involves amputation or limb salvage, with the latter method requiring reconstruction and bone healing. The objective of this study was to develop a murine model of microscopic pulmonary OSA disease that mimics OSA in the post-surgical setting to determine whether the administration of adipose-derived MSCs in the presence of microscopic pulmonary disease would promote pulmonary metastatic OSA progression. We hypothesized that local (surgical site) or IV injection of MSCs would not influence progression of pulmonary metastatic burden once the primary tumor had been removed.

Methods: A syngeneic model of OSA was developed which mimicked the natural disease progression of osteosarcoma. The model built upon a previously published model wherein an orthotopic luciferase-expressing primary OSA tumor was established in tibia of C3H mice. When the tumor-bearing limb was amputated at 16 day post-tumor cell inoculation, there was no gross evidence of metastasis; however, 100% of mice developed metastatic disease following amputation (6). In this experimental model, thirty-four C3H mice had 1x10^6 luciferase-transfected DLM8-M1 osteosarcoma cells injected into the proximal tibia. Primary tumor formation was confirmed in vivo using the PerkinElmer In Vivo Imaging System (IVIS). Fourteen days after tumor inoculation, mice underwent a coxofemoral amputation to remove the primary tumor with wide margins. Mice were randomized to receive adipose-derived C3H MSCs injected either locally at the site of amputation (Group A = 12), systemically through the tail vein (Group B = 13), or were kept as a control group (Group C = 9). Following amputation and MSC injection, development of pulmonary metastases was monitored using IVIS imaging. Mice were sacrificed 24 days following tumor inoculation. At necropsy, lungs were harvested, paraffin embedded, step sectioned, and stained with H&E. The total number of pulmonary metastatic nodules per animal were counted. Area of the total lung tissue per section was measured and sections combined using commercial digital imaging software. Percentage of metastatic area relative to total area was calculated for each group. Data were expressed in mean ± SD. A Kruskal-Wallis test was utilized for analysis of metastatic number and area and a Fisher’s Exact test was used to compare presence or absence of disease. Significance was set at p <0.05. This study was approved by the local Institute for Animal Care and Use Committee and conducted in accordance with academic and national guidelines for the care and use of laboratory animals.

Results: Twenty-six mice displayed evidence of a primary tumor using IVIS imaging (Figure 1); of these, nineteen mice developed pulmonary metastases (Figure 2). A total of n=7 were from Group A, n=5 from Group B, and n=7 from Group C. A significant difference with respect to the presence or absence of metastatic disease (p=1.0) or the mean number of metastatic nodules between groups (p=0.73) was not found between groups (Table 1). Pairwise comparisons also yielded no differences between groups. With respect to the percentage of metastatic area to total area, no significant difference was noted (p=0.13); however, when pairwise comparisons were run, there was a significantly larger percentage of metastatic area in Group A compared to Group C (p=0.048).
Figure 1. Primary osteosarcoma tumor in a C3H
mouse obtained using the IVIS system. Figure 2. Representative pulmonary metastatic nodule at 40x.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Total N per Treatment Group</th>
<th>Percent with Tumor</th>
<th>Mean number of pulmonary metastatic tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>78</td>
<td>3.67 ± 3.67</td>
</tr>
<tr>
<td>Local MSC Injection</td>
<td>10</td>
<td>70</td>
<td>11.35 ± 17.10</td>
</tr>
<tr>
<td>Systemic MSC Injection</td>
<td>7</td>
<td>71</td>
<td>4.93 ± 4.08</td>
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**Discussion:** Osteosarcoma is an aggressive tumor that necessitates large-segment bone resection and reconstruction after surgical removal. The use of MSCs to augment bone healing in limb salvage patients following surgical treatment of OSA is an attractive possibility but the safety of MSC use in sarcoma patients remains a concern. This clinically-relevant animal model of OSA investigated the influence of MSCs in a microscopic disease setting after removal of the primary tumor. In at least one outcome parameter, the use of MSCs increased pulmonary metastatic disease burden following sarcoma removal. Confirmatory studies with larger animal numbers are currently underway.

**Significance:** The use of MSCs to augment bone healing in limb salvage patients following sarcoma resection holds significant therapeutic promise, but further study is required to confirm the safety of their use. Furthermore, the use of a clinically relevant animal model mimicking the natural disease of OSA and its treatment is of paramount importance for translation of results to human populations. This study utilized a novel murine model to study the influence of MSC administration on microscopic pulmonary metastasis following primary osteosarcoma removal. The results indicate some concern that the use of MSCs in the post-primary tumor setting may promote metastatic disease, however, confirmatory studies are required.

**Acknowledgments:**

**References:**

ORS 2014 Annual Meeting
Poster No: 1110