RESECTION OF PRIMARY OSTEOSARCOMA TUMOR ENHANCES THE PULMONARY METASTASIS THROUGH THE ACTIVATION OF ANGIOGENESIS

*Tsunemi, T; **Kaya, M; *Nagoya, S; *Kawaguchi, S; *Yamashita, T; *Wada, T; * Ishii, S
**Dept. of Orthopedic Surg. Sapporo Medical Univ. School of Medicine, Sapporo, Hokkaido, Japan. S-1, W-16, Chuo-ku, Sapporo, , 060-8543, Hookaido, Japan, 81-11-611-2111, Fax: 81-11-641-6026, kaya@sap-cc.go.jp

Introduction

It is well known that more than 50% of the patients with osteosarcoma affected pulmonary metastasis at the time of diagnosis of the primary lesion although such a dormant micrometastasis is often asymptomatic and clinically undetectable until relapse. There are several clinical evidences showing that some of the patients lapse of pulmonary metastasis within months after the removal of the primary lesion. However, little is known about the molecular mechanism of the early relapse of the pulmonary metastasis of osteosarcoma. Thus, to clarify the molecular mechanism of this phenomenon, we have examined whether removal of osteosarcoma can enhance the establishment of pulmonary metastasis. In addition, we assessed that the removal of the primary osteosarcoma tumor enhance the angiogenic activity. Furthermore, we investigated whether s.c. delivery of TNP-470, an angiogenesis inhibitor, inhibited the progression of pulmonary metastasis after the removal of primary osteosarcoma tumor.

Materials

A rat osteosarcoma cell line, MSK-8G, was provided by Department of Oral Surgery, Chiba University, Chiba, Japan. A mouse osteosarcoma cell line, LM 8 was provided by Department of Orthopedic Surgery, Osaka University Medical School, Osaka, Japan TNP-470 was kind gift of Takeda Chemical Industries Ltd. (Osaka, Japan). For pulmonary metastasis assay, MSK-8G rat osteosarcoma cells were inoculated into the tibia of F344 rat and LM8 mouse osteosarcoma cells were inoculated subcutaneously in nude mice. Two weeks after inoculation, the animals were randomly divided into two groups, tumor removal and intact group. In mouse model, sham operation was performed in tumor intact group. The animals were treated with 30mg/kg of TNP-470 or vehicle alone from the day of the tumor resection on every other day. Matrigel plug assay was performed to evaluate the angiogenesis in vivo, Matrigel plug neovascularization assay was performed and Matrigel plug neovascularization was quantitated by measuring the haemoglobin content of the liquefied pellets using Drabkin’s method. VEGF and Endostatin concentrations in the serum of mice were measured by using an enzyme-linked immunosorbant assay kit.

Results

The number of the macroscopic pulmonary metastasis significantly increased in primary tumor removed group compared to the control group. To examine the angiogenic activity in vivo, Matrigel plug neovascularization assay was performed and Matrigel plug neovascularization was quantitated by measuring the haemoglobin content of the pellets using the Drabkin’s method. The haemoglobin concentration of Matrigel plug inoculated into the tumor removed nude mice was significantly higher than those of primary tumor intact group. To investigate the molecular mechanism how the tumor resection promote the angiogenic activity, the plasma levels of known angiogenesis stimulator (VEGF) and inhibitor (Endostatin) that may modulate angiogenesis were evaluated. The VEGF and Endostatin concentration was lower in the serum of the tumor removal group (VEGF, 68.92 ± 1.87 pg/ml; Endostatin, 36.34 ± 2.69 pg/ml) than those in the serum of the tumor intact group (VEGF, 130.09 ± 3.43 pg/ml; Endostatin, 146.19 ± 5.10 pg/ml). Furthermore, the administration of angiogenic inhibitor, TNP-470 resulted in the inhibition of the establishment of pulmonary metastasis after the removal of primary osteosarcoma tumor indicating that the promotion of angiogenic activity due to the removal of primary tumor caused the enhancement of pulmonary metastasis.

Discussion

In this study we have demonstrated that the removal of the primary osteosarcoma tumor have enhanced the establishment of pulmonary metastasis. Furthermore, these malignant progression was due to the activation of angiogenic property. In our experiment model, the removal of primary osteosarcoma tumor resulted in the downregulation of the serum concentration of not only VEGF but also Endostatin. We speculate the mechanism of this phenomenon following; the angiogenic inhibitor, by virtue of its longer half-life in the circulation, reaches the vascular bed of a secondary tumor in excess of angiogenic stimulator escaping from the primary tumor or generated by the secondary tumor. As a result, there is an inhibition of the establishment of pulmonary metastasis. Although we have not examined the status of other angiogenic inhibitory factors, Angiostatin or TGF-β1, these results show that the ability of the primary tumor in our murine osteosarcoma model to suppress the growth of its pulmonary metastasis can be mediated, at least in part, by Endostatin.

The data we have shown here indicates the possibility that the resection of primary osteosarcoma tumor which are considered to be general methods for the treatment of osteosarcoma enhances the establishment of pulmonary metastasis and makes the prognosis of the patients poor. Therefore, the treatments targeted on the inhibition of the angiogenesis after the primary tumor resection will be necessary to improve the prognosis of the patients with osteosarcoma. The data we have shown here that TNP-470 could suppress the progression of pulmonary metastasis after the removal of primary osteosarcoma tumor in animal experiment model means that the administration of anti-angiogenic drugs after the resection of primary osteosarcoma tumor can improve the prognosis of the patients with osteosarcoma.

Acknowledgement

This work was supported in part by a Grant-in-Aid from the ministry of Health and Welfare of Japan (11307026)

Table 1. The number of macroscopic pulmonary metastasis

<table>
<thead>
<tr>
<th></th>
<th>Tumor intact group (n=5)</th>
<th>Tumor resected group (n=5)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat model</td>
<td>2.6±0.6</td>
<td>20.8±3.0</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Mouse model</td>
<td>1.6±0.4</td>
<td>11.2±1.9</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2. Correlation between the serum concentration of VEGF and pulmonary metastasis

<table>
<thead>
<tr>
<th></th>
<th>Tumor intact group (n=5)</th>
<th>Tumor resected group (n=5)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb conc. (g/dl)</td>
<td>1.61±0.1</td>
<td>4.85±0.3</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Poster Session - Tumors - Hall E