**P2X4 receptor antagonist, BAY1797, reduced tumor malignancy in mouse osteosarcoma cells**

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Disclosures: All authors have nothing to disclose.

**INTRODUCTION:**
Most of the causes of death due to the cancer including osteosarcoma is regarded as an invasion of important organs or metastasis to remote organs. Prevention of invasion and metastasis from osteosarcoma cells leads to prolongation of prognosis. Recently, extracellular ATP and its receptors have attracted increasing attention. Extracellular ATP in the tumor microenvironment is associated with tumor cell metabolism, proliferation, and metastasis by driving inflammation and neurotransmission via P2 purinergic signaling. The ATP-gated P2X purine family consists of seven subtypes (P2X1–7 receptors), with some being demonstrated to directly or indirectly regulate tumor proliferation, angiogenesis, and dissemination. We hypothesized that high sensitivity to ATP may upregulate malignant potentials. The purpose of this study is to detect which receptor in seven phenotypes is related to malignancy and to elucidate therapeutic potentials by inhibition of the detected receptor.

**METHODS:**
Mouse osteosarcoma cell lines “Dunn” and its highly metastatic counterpart “LM8” were used. Protein levels of P2X receptors were compared by western blot. P2X4R antagonist, BAY1797, was used for in vitro assay. Cell proliferation potential was determined using an MTS assay. Cell migration activity was assessed using a scratched test. Cell adhesion activity was analyzed using 96-well V-bottom plates. In this assay, centrifugal force is applied to separate adherent from nonadherent cells. The force produced by the centrifugation step results in the accumulation of free or loosely attached cells in the bottom of the V-shaped wells. Nonadherent cells accumulated in the nadir of the V-bottom wells and were quantified. For tumor-transendothelial migration assay, we stained tumor cells with calcein AM and thereafter cells are seeded above the endothelial cell monolayer on the top-side of the transwell insert. The number of cells labeled with calcein AM that migrated to the bottom of the transwell insert is calculated by using a fluorescence microscope.

**RESULTS:**
From the western blot analysis, protein levels of P2X4 receptor were expressed higher in LM8 than in Dunn. We proceeded further study using P2X4R antagonist, BAY1797. Cell proliferation potential was measured at the intervals of 24 and 48 hours following the administration of BAY1797(0.1, 1, 10 μM). At the intervals of 24 hours, BAY1797 indicated 4% reduction (p<0.005) at 10 μM compared to control. At the intervals of 48 hours, BAY1797 indicated 7% reduction (p<0.05) at 1 μM and 25% reduction (p<0.005) at 10 μM compared to control. Migration activity was assessed by scratched test. Images were acquired at 0, 24, 48, 72, 96, and 132 h after scratch and analyzed using ImageJ software. Migration rate was significantly inhibited by 1 μM of BAY1797 compared to control(Figure 1). Cell adhesive activity was evaluated by nonadherent cells by V-bottom plates. Cell adhesive activity was significantly suppressed by 10 μM of BAY1797(Figure 2). Transendothelial migration activity of LM8 cells was significantly inhibited by 10 μM of BAY1797 compared to control (Figure 3).

**DISCUSSION:**
BAY1797 reduced the proliferation, migration, adhesion, and transendothelial migration activity of LM8 cells in vitro by inhibition of the P2X4R. Our results provide the first evidence that P2X4R antagonist administration reduces tumor malignancy of osteosarcoma. The P2X4R antagonist might be a novel target for the therapy of osteosarcoma.

**SIGNIFICANCE/CLINICAL RELEVANCE:** This study showed the possibility of molecular targeted therapy for osteosarcoma by P2X4R antagonist.

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**Figure 1**

Migration rate (%)

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<th>Time/hours</th>
<th>0h</th>
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<th>48h</th>
<th>72h</th>
<th>96h</th>
<th>132h</th>
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</tr>
<tr>
<td>1 μM</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10 μM</td>
<td></td>
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<td>**</td>
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</table>

*p<0.05, **p<0.005

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**Figure 2**

Cell adhesion (%)

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*p<0.005

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**Figure 3**

Immunostained cell number

<table>
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<th>LM8</th>
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*p<0.005