Decorin suppresses the bone metastasis in breast cancer cell line

Kentaro Araki, Hiroki Wakabayashi, Ken Shintani, Akihiko Matsumine, Katsuyuki Kusuzaki, Atsumasa Uchida
Orthopaedic Surgery, Mie University Graduate School of Medicine, Tsu, Japan
whiroki@clin.medic.mie-u.ac.jp

Introduction: Decorin, the member of small leucine-rich proteoglycans, is involved in a number of cellular processes including matrix assembly, fibrillogenesis, and the control of cell proliferation [1]. In this study, we investigated the role of decorin in suppressing tumor aggressiveness and bone metastases.

Materials and Methods: The human breast cancer cell line MDA-MB-231 was cultured in DMEM supplemented with 10% FBS.

Five-week-old female BALB/c mice were used for all in vivo experiments.

We established two cell lines. One is MDA-DCN which stably expressed human decorin and another is MDA-EV (empty vector) established as a control. The hDCN gene was amplified by PCR. After digestion with BamHI and EcoRI restriction enzymes, hDCN gene was inserted into the multiple cloning site of p3XFLAG-CMV-14 expression vector. We used p3XFLAG-CMV-9 expression vector as a control vector. Cells were transfected using Lipofectamine.

Western blots were performed using total proteins from each case to analyze the expression of decorin.

For proliferation assay, three cell lines (MDA-231, MDA-EV, MDA-DCN) were seeded in duplicate at a density of 2.0 x 10⁴ cells/well in 24-well plates. Cells were trypsinized and counted everyday or every two days.

For adhesion assay, plates were coated with Matrigel at 37°C. Cells (1 x 10⁵) were seeded in serum-free DMEM medium and incubated at 37°C with 5% CO2 for 30 minutes. Cells were washed with PBS and stained and examined by microscopy. Five random fields in each well were photographed at x200 magnification, and the total number of adhered cells in every field was counted.

Boyden chambers were used for in vitro migration and invasion assays. Cells were added to the upper compartment of the chamber. The two compartments were separated by a polyethylene terephthalate membrane. In simple migration studies, the filter was not coated with Matrigel. The lower compartment contained DMEM and fibronectin as a chemo-attractant. Cells were added to the upper chamber. The chambers were incubated for 22 hours and cells on the upper surface of the membrane were thoroughly removed. Quantitation of the result of an in vitro migration and invasion study was performed by staining membranes and counting the average number of cells in a high power field.

For making intracardiac experimental metastasis model, cells (1 x 10⁵ for each cell) were suspended in 0.1 ml PBS, and injected into the left ventricle of 5 week-old female nude mice.

Development of bone metastasis was monitored by X-ray radiography once a week. Around knee joints, radiolucent areas were detected 5 weeks after inoculation. Measurement of osteolytic lesions was made on femur and tibia per mouse. Mice were sacrificed five weeks after inoculation. Bilateral hindlimbs were examined. We performed histomorphometrical analysis of tumor burden in the metastatic tumors in the distal femoral and proximal tibial metaphyses of both hindlimbs, using longitudinal sections stained with H&E. Tumor measurements were made in the intraosseous and extrasosseous regions of distal femur and proximal tibia on the central section of the tumor. The measurement area in each bone was 1.5 mm beginning 100 μm below the growth plate. Dates of tumor burden are shown as tumor area/mm².

Results: The MDA-DCN and MDA-EV had morphology similar to MDA-231.

The proliferation assay exhibited a significant decrease of MDA-DCN proliferation rate compared with MDA-231 and MDA-EV in 96 and 144 hours after seeding tumor cells. The adhesion assay showed no significant difference between MDA-DCN and others. And we observed that migration and invasion were significantly reduced in MDA-DCN cells relative to MDA-231 and MDA-EV.

Around the knee joints of mice injected with MDA-231 or MDA-EV cells, osteolytic bone metastases appeared at days 14 postinoculation and progressively increased to 0.11 and 0.12 points of incidence, respectively. In contrast, in mice receiving MDA-DCN cells, no osteolytic lesions were detected until day 35, with 0 points of incidence at sacrifice. Similar results were observed in osteolytic area per mouse inoculated three cell lines respectively.

In bone metastasis, tumor cells of three cell lines had similar morphological feature. Histological examination of femurs and tibias injected with MDA-231 and MDA-EV cells showed wide tumor burden and trabecular bone erosion. Furthermore, the frequency of bone metastasis in MDA-DCN injected mice was lower than that in vehicle-treated mice.

Table 1: Histomorphometric analysis of intraosseous and extrasosseous tumor burden per mouse of tumor cells growing in tibia and femur of 5-week-old female nude mice

<table>
<thead>
<tr>
<th>Tumor burden</th>
<th>Bone surface</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-231</td>
<td>2.19±0.23</td>
<td>0.01</td>
</tr>
<tr>
<td>MDA-EV</td>
<td>2.08±0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>MDA-DCN</td>
<td>1.19±0.18</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

= 12 per experimental group. P values represent MDA-DCN versus others (MDA-231 and MDA-EV) in tumor burden.

Although, there is no statistical significance because of the difference in the number of metastasis among three groups, histomorphometric analysis in sections histochemically stained for the osteoclast marker TRACP showed a significant decrease of osteoclast surface/bone surface in mice injected with MDA-DCN compared with MDA-231, MDA-EV cells injected control.

Discussion: Gene therapy of established tumor xenografts using decorin-expressing adenovirus vectors causes a growth inhibition of various tumors [1-3] and prevents metastatic spreading of a breast carcinoma orthotopic tumor model [4]. In this report, we demonstrate that transient transgene expression of virus-containing decorin causes a significant growth inhibition of breast cancer tumor xenografts.