Effects of Ad-p27mt Gene Transfer on the Expression of Bax, Bcl-2, VEGF and MMP-9 in the Transplanted Liver Tumors in Nude Mice

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Summary: In this study, the mechanism by which Ad-p27mt inhibits the growth, invasion and metastasis of transplanted liver tumor was studied by examining the effects of Ad-p27mt gene transfer on the expression of Bax, Bcl-2, VEGF and MMP-9 in the transplanted liver tumors in nude mice. The model of transplanted hepatic tumor was established in nude mice. The mice were then divided into three groups, which were injected with PBS, Ad-LacZ and Ad-p27mt and the growth of the transplanted liver tumor was observed. The expressions of P27, Bax and Bcl-2 proteins were detected by Western blotting and the expressions of VEGF and MMP-9 were immunohistochemically determined. Our result showed that the tumor size, expressions of Bax, Bcl-2 proteins, VEGF and MMP-9 were all lower than those in PBS and Ad-LacZ groups and the differences were statistically significant (P<0.05). Our study suggested that Ad-p27mt could inhibit the growth, invasion and metastasis of hepatic cancer by lowering the expressions of VEGF and MMP-9.

Key words: Ad-p27mt; hepatocellular carcinoma; transplanted tumors

Hepatocellular carcinoma (HCC) is a common malignancy of the gastrointestinal tract. With most cases of HCC, the patients have developed metastasis when their clinical symptoms become apparent, which renders surgical cure virtually impossible. Recently, some advances have been made in the gene treatment of liver carcinoma and p27mt gene is a newly identified cancer-inhibiting gene[1]. In this study, the mechanism by which Ad-p27mt inhibits the growth, invasion and metastasis of transplanted liver tumor was studied by examining the effects of Ad-p27mt gene transfer on the expression of Bax, Bcl-2, VEGF and MMP-9 in the transplanted liver tumors in nude mice.

1 MATERIALS AND METHODS

1.1 Materials

BALB/c mice of both sexes were purchased from the Hubei Provincial Center of Experimental Animals, Wuhan, China. The HCC cell line (SMMC-7721) was bought from Institute of Biochemistry and Cell Biology, Shanghai, China. Mouse anti-human p27 monclonal antibody, rabbit anti-human Bax polyclonal antibody, mouse anti-human Bcl-2 polyclonal antibody and S-P kit for immunohistochemistry (instant type) were procured from Wuhan Boster Biological Technology, Ltd. Company, Wuhan, China. Recombinant adenoviral vector (Ad-p27mt) was provided by the Institute of Clinical Research, Hubei Medical University, Shiyan, China. Ad-lacZ virus was a kind gift from Professor Shaoyong XU of Hubei Medical University. Rabbit anti-human VEGF polyclonal antibody, rabbit anti-human MMP-9 polyclonal antibody were all obtained from Shanghai MajorBio Technology Co. Ltd., Shanghai, China.

1.2 Methods

1.2.1 Cell Culture

SMMC-7721 cells were incubated in DMEM at 37°C in 5% CO2 and passaged repeatedly to obtain 30 bottles of the cells. The cells at logarithmic phase were then digested with 0.25% Trypsin and washed with PBS and re-suspended for later use.

1.2.2 Establishment of Model of Transplanted Hepatic Tumor

About 0.2-mL PBS containing 5×106 SMMC-7721 cells were subcutaneously transplanted into the back skin of BALB/c nude mice aged 4–6 weeks (n=30). After the transplantation, the animals were maintained in IVC cages at SPF laboratory of the aforementioned center of experimental animals. When the tumors grew to the size of 0.5 cm (diameter), the animal model of transplanted hepatic tumor was deemed successful. Then the animals were divided into three groups. In Ad-p27mt group, 0.1 mL of Ad-p27mt was injected into the tumor; in Ad-LacZ group, which served as a positive control, 0.1 mL Ad-LacZ was injected into the tumor; in blank control group, 0.1 mL PBS was injected into the tumor. In each group, three injections were given at an interval of two days. The animals were sacrificed on the 21st day after the last injection. Afterwards, the whole tumor was harvested, washed with PBS, with

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some tumors being cryo-sectioned immediately after PBS washing. Some tumors were stored at –80°C for later use.

1.3 Items for Observation

1.3.1 Size of Tumors The vertical and horizontal diameters (a and b) of the tumor were measured on day 7, 14 and 21 and the volume of the tumor (V) was calculated by the following formula: \( V = \frac{\pi ab^2}{6} \).

1.3.2 Expression of P27, Bax and Bcl-2 Proteins Western blot analysis was employed to detect the expression of P27, Bax and Bcl-2 proteins in Ad-27mt and Ad-LacZ groups.

1.3.3 Expression of VEGF and MMP-9 The VEGF and MMP-9 expressions was immunohistochemically detected. The presence of brown-yellow particles was taken as an indication of positive expression. For each sample, 5 visual fields (400×) were randomly selected and 200 cells were counted. The result was deemed as negative (–) when less than 5% of the cells were positively stained. The result was respectively taken as positive (+), strongly positive (+++) and very strongly positive (++++) when 5%–15%, 16%–25% or more than 25% of the cells were positively stained.

1.4 Statistical Analysis

The variable data were expressed as \( \bar{x} \pm s \) and processed and intra-group differences were processed by the Student’s \( t \) test. \( \chi^2 \) test was used for the process of categorical data. A \( P \) less than 0.05 was considered to be statistically significant. SPSS software package (Version 13.0) was used for statistically analysis.

2 RESULTS

2.1 Effect of Ad-p27mt on the Growth of Transplanted Hepatic Tumors

Ad-p27mt intratumoral injection started on the 5th day after the successful establishment of transplanted liver tumor model. The volume of the transplanted tumors was measured on day 7, 14 and 21, respectively. (table 1, fig. 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time (d)</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad-p27mt</td>
<td></td>
<td>0.63±0.07</td>
<td>0.70±0.64*</td>
<td>1.30±0.29*</td>
</tr>
<tr>
<td>Ad-LacZ</td>
<td></td>
<td>0.75±0.07</td>
<td>2.74±0.94</td>
<td>4.19±1.01</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.74±0.11</td>
<td>2.58±0.53</td>
<td>4.20±0.58</td>
</tr>
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</table>

\( P<0.05 \) as compared with the control group; \( ^* P<0.05 \) as compared with Ad-LacZ group

Fig. 1 Comparison among the tumor size of three groups of BALB/c nude mice with transplanted liver tumor 21 days after the establishment of the transplanted liver tumor

2.2 Expression of P27, Bax and Bcl-2 Proteins

The expression of P27, Bax and Bcl-2 proteins in Ad-p27mt and Ad-LacZ groups was detected by Western blotting. P27 protein was found to be highly expressed in Ad-p27mt group and only very low P27 protein expression was detected in Ad-LacZ and blank control (fig. 2). It was also found that the ratio of Bax/Bcl-2 was raised with the increased P27 protein expression.

2.3 Expression of VEGF and MMP-9

The animals were sacrificed and the transplanted liver tumors were harvested 21 days after the intratumoral injection. Then, the expression of VEGF and MMP-9 was determined by S-P immunohistochemical method. The results showed that the expressions of VEGF and MMP-9 were low in Ad-27mt group while it was highly expressed in Ad-LacZ and control group and the difference was statistically significant \(( P<0.05)\) (table 2, 3 and fig. 3, 4).

<table>
<thead>
<tr>
<th>Groups</th>
<th>VEGF</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
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<td>5</td>
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<td>1*</td>
</tr>
<tr>
<td>Ad-LacZ</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

\( P<0.05 \) as compared with the control group; \( ^* P<0.05 \) as compared with Ad-LacZ group