Author's response to reviews

Title: Dynamic distribution and expression in vivo of human interferon gamma gene delivered by adenoviral vector

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Author's response to reviews: see over
Dear Dr. Alam:

Thank you for your review of our manuscript. We also appreciate the advice of the reviewer, Dr. Guillermo Mazzolini and we have revised the manuscript according to his suggestions. We now illustrate the anti-tumor effects of intratumoral injection of Ad-IFNγ using the CNE-2 tumor model (Zhu YH et al., unpublished data) in Supplementary Figure 1 and in the discussion. The Ad copies in tumors and livers were shown in detail in Table 1.

Thank you again for your reviews and advice.

Response to Reviewer

The following is our point-by-point response to the reviewer.

Reviewer Dr. Guillermo Mazzolini:

1. If the authors have the information regarding antitumoral effects of the vector why not to include this as a new figure. This information will increase the strength of the paper.

Response:

We now illustrate the anti-tumor effects of intratumoral injection of Ad-IFNγ using the CNE-2 tumor model (Zhu YH et al., unpublished data) in Supplementary Figure 1 and in the discussion.

Consistent with the anti-tumor effects of r-hu-IFNγ, Ad-IFNγ produced a significant,
dose-dependent inhibition of tumor growth in the CNE-2 model. Intratumoral administration of Ad-IFNγ at doses of 1×10⁹ and 5×10⁸ pfu/week for three weeks led to inhibition rates of 67.9% and 58.64%, respectively (Supplementary Fig. 1, Zhu YH, et al., unpublished data).

Supplementary Fig. 1. Antitumor activity of Ad-IFNγ on CNE-2 xenografts was assessed by tumor weight. Female athymic nude mice were inoculated s.c. in the scapular region with 2×10⁶ CNE-2 cells in 100μl sterile PBS. When tumors reached a volume of 30 to 40mm³, animals were randomly assigned into 6 experimental groups of 6-7 animals: Ad-IFNγ (1×10⁹, 5×10⁸ or 1×10⁸ pfu/week), 1×10⁹ pfu/week of Ad-LacZ, 1×10⁶ IU/kg/d of r-hu-IFNγ or PBS alone was intratumorally injected. Mice were killed after 3 weeks of treatment and tumors were resected and weighted. Columns, average weight of tumor from 6-7 mice; bars, SD. *, p < 0.05, compared with the PBS-treated and the Ad-LacZ group.

Supplementary Fig. 1.
2. The authors evaluated the Ad copies in tumors and livers by hexon DNA PCR, however, it would be of interest to show this data in a figure or a table.

Response:

In our study, Ad-IFN\(\gamma\) (diluted with 0.9% NaCl to 100 \(\mu\)l) or vehicle was injected into the center of the tumor. Adenovirus copies in tumors and livers were measured by hexon DNA PCR. The Ad copies in tumors and livers were shown in detail in Table 1.

Table 1. Adenovirus copies in tumors and livers were measured by hexon DNA PCR.

The dose of injection was 1 \(\times\) 10\(^{10}\) VP/tumor. Data represent mean \(\pm\) SD of three mice. Representative results from two independent experiments are shown.

<table>
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<th>day</th>
<th>tumor (copies/(\mu)g tissue DNA)</th>
<th>liver (copies/(\mu)g tissue DNA)</th>
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<tr>
<td>1</td>
<td>897927.2 (\pm) 188201.2</td>
<td>206274.7 (\pm) 67269.5</td>
</tr>
<tr>
<td>2</td>
<td>645995.1 (\pm) 76137.4</td>
<td>168531.6 (\pm) 46526.4</td>
</tr>
<tr>
<td>3</td>
<td>289441.2 (\pm) 75017.3</td>
<td>109217.2 (\pm) 5478.3</td>
</tr>
<tr>
<td>5</td>
<td>197677.1 (\pm) 220320.1</td>
<td>90712.8 (\pm) 68176.5</td>
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<td>169882.6 (\pm) 69067.4</td>
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