Author's response to reviews

Title: Intratumoral Delivery of IL-18 Naked DNA Induces T-Cell Activation and Th1 Response in a Mouse Hepatic Cancer Model

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Author's response to reviews: see over
Revision Report

Title: Intra-tumoral delivery of IL-18 naked DNA induces T-cell activation and Th1 response in a mouse hepatic cancer model

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Thank you for the opportunity to provide a revision of my manuscript. We edited some sentences by your comments following list of revision. We really agreed that some experimental data have to be repeated and improved as you mentioned. But we’re sorry that we couldn’t replace data in this article. Because we feel that the tumor cell line studied herein is adequate to represent a model closely to conditions known from human colorectal cancer metastasis model. Moreover, we focused on possibility of clinical trial in human and that’s why we performed abdominal operation in order to inject plasmid DNA directly in tumor site. In intracellular staining data, CD4⁺CD69⁺IFN-γ T cells were consisting of small population in total CD4⁺ T cells. Then, we couldn’t have an idea of sorting splenic CD4⁺ T cells only. We attached the JPG file to explain the tumor development for reference. I’m very thankful for your detailed review of my paper and appreciate the helpful comments.

List of Revision

Abstract
results
: 2nd line
in dose dependent manner → removed

Background
: 8st line
correct reference [4], [5]
: 20st line
We report here antitumoral effects of nonviral vector encoding murine IL-18 plasmid DNA in established CT26 liver tumors.

→ We report here the antitumoral effects by direct intratumoral injection of nonviral plasmid vector encoding murine IL-18 plasmid DNA in established CT26 liver tumors.
Methods:

Tumor model and therapeutic protocol

Colon carcinoma was established in the liver by intrahepatic implantation of $1 \times 10^5$ cells in the left lateral lobe of 6- to 8-week-old male BALB/c mice. Seven days after implantation, mice with liver tumors were treated with pCEP4 control vector or mouse IL-18 in pCEP4 at various doses (10, 25, and 50 ug)

Colon carcinoma was suspended for implantation at $1 \times 10^5$ cells/50 μl saline. Colon carcinoma was established in the left lateral lobe of 6- to 8-week-old male BALB/c mice by an abdominal operation. Treatments began 7 days later, when tumor results in a defined solid tumor growth within the injected lobe. For intratumoral injections of naked DNA, DNA (10, 25, and 50 μg) was diluted in 50 μl saline, and then injected into the parenchyma of the lower surface of the left liver lobe via insulin syringes (31 gauge, 0.8-inch needles; Becton Dickinson, Franklin Lake, NJ). Mice were sacrificed at 1, 4, 7, and 14 days after DNA treatments and then tumor growth was monitored by measuring liver weight [13].

Results and discussion

levels of transgene expression in treated tumor site

A dose-dependent antitumor response against CT26 was noted in group receiving pIL-
Antitumoral response of tumor-bearing mice when treated with 50 \( \mu g \) of DNA was significantly greater than that of the tumor-bearing mice treated with 10- or 25-\( \mu g \) DNA groups.

The result suggest that high level of IL-18 modulates immune cell population of T cells and NK cells around the time of tumor regression.

Other groups have also reported that IL-18 has been shown to up-regulate ICAM-1 expression on monocytes and on T cells, providing further mechanisms whereby IL-18 can promote T cell recruitment [14-17]. These data, together with the above data about kinetic of immune cell population, suggested that about ~2.5 fold higher concentration of IL-18 modulates immune cell population of T cells and NK cells around the time of tumor regression.

Intratumoral injection of mIL-18 plasmid DNA elevated IFN-\( \gamma \) production by splenocytes.

However, in our studies with tumor-bearing mice revealed that IL-18 plasmid intratumoral injection does not prevent development of CT26 tumors although such augmentation of T cells and maintenance during of IFN-\( \gamma \) level during 2 weeks appeared to reject tumors. Thus, direct cytokine gene transfer may have a therapeutic potential but expression of cytokine gene may be elevated and sustained significantly during tumor development. Other reports show that the cotransfection of ICE and pro-IL-18 cDNA was superior to pro-IL-18 cDNA alone and resulted in enhanced bioactivity of IL-18. Thus dual transfection of pro-IL-18 and ICE cDNA results in secretion of more bioactive IL-18 protein than that of pro-IL-18 cDNA alone [24].

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