Author’s response to reviews

Title: Induction of protective and therapeutic anti-pancreatic cancer immunity using a reconstructed MUC1 DNA vaccine

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Author’s response to reviews: see over
1. **Induction** of protective and therapeutic anti-pancreatic cancer immunity using a reconstructed MUC1 DNA vaccine

   **Induction** of protective and therapeutic anti-pancreatic cancer immunity using a reconstructed MUC1 DNA vaccine

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4. MUC1-VNTR DNA vaccine was produced by cloning one repeat of VNTR and

   by inserting the cloned gene into the pcDNA3.1.

   MUC1-VNTR DNA vaccine was produced by cloning one repeat of VNTR and

   inserting the cloned gene into the pcDNA3.1.

5. The DNA vaccine pcDNA3.1-VNTR could raise specific CTL activity and this

   CTL activity was MUC1-VNTR specific.

   The DNA vaccine pcDNA3.1-VNTR could raise specific CTL activity and this CTL

   activity was MUC1 specific.

6. In the preventive group, the mice survival time was significantly longer in vaccine

   subgroup than the control groups

   In the preventive group, the mice survival time was significantly longer in the vaccine

   group than in the control groups

7. In the preventive and therapeutic group, the tumor size was significantly less in

   vaccine subgroup than the control groups (P<0.05).

   In the preventive and therapeutic group, the tumor size was significantly less in the

   vaccine group than in the control groups (P<0.05).

8. The MUC1 DNA vaccine pcDNA3.1-VNTR could significantly induce MUC1

   specific CTL response; and has distinctive prophylactic effect on tumor

   panc02-MUC1; and also can inhibit the tumor growth in vivo. This vaccine may

   be used as a new strategy on pancreatic cancer.

   The MUC1 DNA vaccine pcDNA3.1-VNTR could significantly induce MUC1

   specific CTL response; and had distinctively prophylactic and therapeutic effect on

   tumor panc02-MUC1; and also could greatly inhibit the tumor growth in vivo. In

   conclusion, this vaccine might be used as a new adjuvant strategy against pancreatic
cancer.

9. **Keyword:** MUC1; pancreatic cancer; DNA vaccine
Delete.

9. which consist of 20 amino acids (GVTSAPDTRPAPGSTAPPAAH) and are the most specific epitope for tumor immunotherapy\(^4,\,5\).
which consists of 20 amino acids (GVTSAPDTRPAPGSTAPPAAH) and is the most specific epitope for tumor immunotherapy [4, 5].

10. **In addition,** von Mensdorff-Pouilly et al. reported that breast carcinoma patients who had natural humoral responses against
Von Mensdorff-Pouilly et al. reported that breast carcinoma patients who had natural humoral responses against
11. and most of the vaccine was used for prevention of the tumor, few of them were used for therapy.

**Furthermore,** most of the vaccines were used for prevention of the tumor; and few of them were used for tumor therapy.

12. non-radioactive lactate dehydrogenase (LDH)-releasing assay

non-radioactive lactate dehydrogenase (LDH)-releasing assay

13. Serum samples were obtained from mice by retro-orbital bleeding 5 days
Serum samples were obtained from mice via retro-orbital bleeding 5 days

14. **Figure**

**Figure**

15. The C57BL/6 mice have been bred in the Animal Lab center (Medical school of Jiaotong University, Shanghai).
The C57BL/6 mice were bred in the Animal Lab center (Medical school of Jiaotong University, Shanghai). **The facility was approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all procedures were carried out in accordance to the Guidelines and Regulations for Use and Care of Animals in Fudan University.**

16. To study the protective immune response of pcDNA3.1-VNTR vaccine against pancreatic cancer, the mice were **given** DNA vaccine three times before the mice were challenged with \(1 \times 10^6\) panc02-MUC1 or panc02.
To study the protective immune response of pcDNA3.1-VNTR vaccine against pancreatic cancer, the mice were **inoculated with** DNA vaccine three times before the mice were challenged with \(1 \times 10^6\) panc02-MUC1 or panc02.
17. Figure 3 has been changed into Figure 3A and Figure 3B
18. Figure 4 has been changed into Figure 4A and Figure 4B
19. Figure 7 has been changed into Figure 7A and Figure 7B
20. Figure 8 has been changed into Figure 8A and Figure 8B
21. Any kind of vaccine finally should have the effect to cure the tumor. 
   **Theoretically**, any kind of vaccine finally should have the effect to cure the tumor.

   21. Our data **suggest** that similar immunization strategies **may** be used in 
   pancreatic cancer patients with over-expression of MUC1 for the treatment of early 
   cancers or the eradication of minimal residual lesions.

   Our data **suggested** that similar immunization strategies **might** be used in pancreatic 
   cancer patients with over-expression of MUC1 for the treatment of early cancers or 
   the eradication of minimal residual lesions.

22. The references have been listed in the new version.
To the reviewers

Reviewer: Bo Zhu

“In the experiments of tumor protection, in the materials and methods the authors divided three groups including PBS, empty plasmid pcDNA3.1 and pcDNA3.1-VNTR, and then challenged by panc02-MUC1 tumor cells after vaccination. However, in Fig.3 and Fig.4 there is four groups including PBS, empty plasmid pcDNA3.1, pcDNA3.1-VNTR and pcDNA3.1-VNTR-panc02. It is confusing! Does pcDNA3.1-VNTR-panc02 mean pcDNA3.1-VNTR vaccination and panc02 tumor cell challengment?

In the experiments of tumor therapy, in the materials and methods they used both panc02 and panc02-MUC1 tumor cells to make tumor model, and they vaccinated with PBS, empty plasmid pcDNA3.1, pcDNA3.1-VNTR for these two groups of tumor-bearing mice individually. But in Fig.7 and Fig.8, they express the results of four groups of PBS, empty plasmid pcDNA3.1, pcDNA3.1-VNTR and pcDNA3.1-VNTR-panc02. It is confusing as well! As referee understands, they use panc02-bearing model to prove the specificity of MUC1 for the vaccination. They should show the results of panc02 and panc02-MUC1-bearing mice individually.”

Thanks to the reviewer Bo Zhu.

Revision: In the revised article, the results of panc02 and panc02-MUC1-bearing mice have been shown individually according to the reviewer’s suggestion(Figure3, Figure4, Figure7 and Figure8).
Thanks to reviewer Zhiguo Liu.

1. “Reference list and Figure 6 have been missed”
   In the revised article, these two mistakes have been revised.

2. “why authors need to produce a MUC1 over-expressed pancreatic cancer cell line, rather than choosing a proper cell line, it there any major difference between mouse and human MUC1, which made them have to establish an artificial over-expression cell line? The authors have not explained.”
   **Explanation:** Human pancreatic cancer cell line capan-2 and AsPC-1 do express MUC1 protein; however these two cell lines only could form tumors on the nude mice. To investigate the protective and therapeutic ability of the DNA vaccine pcDNA3.1-VNTR, we must establish pancreatic cancer on the normal C57BL/6 mice which have normal immune system. So we used the murine pancreatic cancer cell line panc02 in our research.

   However, there was no murine pancreatic cancer cell line that could express MUC1. And over 80% of the human pancreatic cancer could express MUC1, and this protein was consisted of 20-50 VNTR. So we produced a MUC1- over-expressed pancreatic cancer cell line which would be the same to the human pancreatic cancer. Thirdly, the human MUC1 is homological with the murine MUC1.

3. “By using such an artificially over-expressed cell line, there are several possible issues arisen, one is the expressed MUC1 would mainly located at cytoplasm since only a regular pcDNA3 vector was used, how could the CTL recognized the intra-cellular MUC1?”
   **Explanation:** Indeed the expressed MUC1 maybe located at cytoplasm since only a regular pcDNA3 vector was used, however in our research we found out that the MUC1 was mainly expressed on the surface of the monoclonal cell lines as shown in the immunostaining analysis (Figure2) and this could be recognized by the CTL (Figure6).

4. “The second is an aberrantly expressed foreign molecules would tend to induce dramatic immune response from the host itself, no wonder in figure 3, the tumor volume formed by parental Cell line would be much larger compared with PBS and pcDNA3.1 controls. Therefore any comparison with such bias will be very difficult and misleading.”
   **Explanation:** Although the human MUC1 is homological with the murine MUC1, the human MUC1 is still a foreign protein and might induce immune response from the host itself. However in these 3 groups: pcDNA3.1-VNTR, pcDNA3.1 and PBS, the difference is great; and this might show that the pcDNA3.1-VNTR vaccine could induce protective and therapeutic anti-pancreatic cancer immunity and it was MUC1-specific. Furthermore the cytotoxicity assay showed that the pcDNA3.1-VNTR could induce MUC1-specific CTL and did kill the panc02-MUC1 cells.
I admit that there were many articles about MUC1 vaccine, but few of them investigate the therapeutic ability of the vaccine especially in pancreatic cancer, while we studied the therapeutic effect of the MUC1 vaccine and indeed it had therapeutic effect to the pancreatic cancer. As we all know that the pancreatic cancer is the most lethal disease, even the radical surgery could not prolong the survival time of the patients. Therefore any new useful strategy to this disease might be a new adjuvant method. In our research, the pcDNA3.1-VNTR vaccine indeed induced protective and therapeutic anti-pancreatic cancer immunity which was MUC1-specific and this vaccine might be used as an adjuvant therapy for the pancreatic cancer patients. Here we hope that the editorial could give us a chance to share our work with those who are concerned about the pancreatic cancer.