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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Version: 3 Date: 6 June 2009

Author’s response to reviews: see over
Dear editor:

Thank you for your hard work. Here is the point-by-point description of the changes made:

1. Reference changes: The reference list have contain all authors’ names, if there are 10 or below. Full and accurate details for each reference have been added.
2. The figure title or figure number has been removed in figure file: figure3, figure4, figure7, figure 8.
3. In the figure legend, the “figure3A, figure3B, figure4A, figur4B, figure7A, figure7B, figure8A, and figure8B” have been changed into “A and B”.
4. “All authors read and approved the final manuscript.” ---This sentence has been added in the author’s contributions section.
5. Abstract: “Active immunotherapy targeting MUC1” is changed into “Active immunotherapy that targets MUC1”.
6. Abstract: “MUC1-VNTR DNA vaccine” is changed into “MUC1-various tandem repeat units (VNTR)”.
7. Abstract: “the female C57BL/6 mice were immunized by vaccine” is changed into “female C57BL/6 mice were immunized with the vaccine”.
8. Abstract: “and attacked by panc02-MUC1” is changed into “and challenged with panc02-MUC1”.
9. Abstract: “The tumor size and mice survival time were compared within these groups” is changed into “The tumor size and the survival time of the animals were compared between these groups.”
10. Abstract: “The DNA vaccine pcDNA3.1-VNTR could raise specific CTL activity and this CTL activity was MUC1 specific” is changed into “The DNA vaccine pcDNA3.1-VNTR could raise cytotoxic T lymphocyte (CTL) activity specific for MUC1.”
11. Abstract: “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” is changed into “The MUC1 DNA vaccine pcDNA3.1-VNTR could induce a significant MUC1-specific CTL response; and had both prophylactic and therapeutic effect on panc02-MUC1 tumors. This vaccine might be used as a new adjuvant strategy against pancreatic cancer.”
12. Background: “Therefore new approaches are necessary to improve the outcome of patients suffered from pancreatic cancer” is changed into “Therefore new approaches are necessary to improve the outcome of patients suffering from pancreatic cancer.”

13. “Immunotherapy has become a feasible tumor specific therapy nowadays” is changed into “Immunotherapy has recently become a feasible tumor specific therapy.”

14. “MUC1 is a tumor-associated antigen which over-expressed in pancreatic adenocarcinomas” is changed into “MUC1 is a tumor-associated antigen that is over-expressed in pancreatic adenocarcinomas”.

15. “In addition, cytotoxic T lymphocytes (CTLs) specific to MUC1 have also been found in breast, pancreatic, and ovarian carcinoma patients” is changed into “In addition, MUC1-specific cytotoxic T lymphocytes (CTLs) have also been found in breast, pancreatic and ovarian carcinoma patients”.

16. “However, although MUC1-specific antibodies and/or CTLs were detected in some patients, none of them were adequate to generate effective anti-tumor immunity” is changed into “Although MUC1-specific antibodies and/or CTLs were detected in some patients, they were not adequate to generate effective anti-tumor immunity”.

17. “it is necessary to develop new vaccination protocols to induce strong anti-tumor immune responses and apply for pancreatic cancer therapy” is changed into “it is necessary to develop new vaccination protocols to induce strong anti-tumor immune responses that are applicable for the therapy of the pancreatic cancer.”

18. “An optimized Kozak and hMCP-1 sequences” is changed into “An optimized Kozak sequence and an hMCP-1 sequence”.

19. “The panc02 (kindly provided by MD Anderson Cancer Center) is a highly tumorigenic murine pancreatic tumor cell line with ductal morphology that was derived in 1984 from a methylcholanthrene-induced tumor growing in a C57BL/6 female mouse” is changed into “The panc02 is a highly tumorigenic murine pancreatic tumor cell line with ductal morphology that was derived in 1984 from a methylcholanthrene-induced tumor growing in a C57BL/6 female mouse (kindly provided by MD Anderson Cancer Center)”.

20. “It has been shown to produce rapidly growing local tumors after s.c.
inoculation” is changed into “It produces rapidly growing local tumors after s.c. inoculation.”

21. “Cytotoxicity(%)=(\[E-Se-St]\)/([Mt-St]) \times 100\%” is changed into “Cytotoxicity(%)=[(E-Se-St)/(Mt-St)] \times 100\%”.

22. “The cell line panc02-MUC1-C was named as pan02-MUC1 and used in the later research while the cell line transfected with pcDNA3 was named as panc02-Neo (brief in panc02)” is changed into “The cell line panc02-MUC1-C was named pan02-MUC1 and used in subsequent experiments, while the cell line transfected with pcDNA3 was named panc02-Neo (abbreviated as panc02)”.

23. “As show in Figure4A, the mice immunized with pcDNA3.1-VNTR showed a more effective protection against the panc02-MUC1 tumor cells than the mice in the pcDNA3.1 and PBS group, because the mice in this group survived longer in comparison with the mice in the other two groups” is changed into “As show in Figure4A, the mice immunized with pcDNA3.1-VNTR were protected better against the panc02-MUC1 tumor cells than the mice in the pcDNA3.1 and PBS groups, because the mice in this group survived longer in comparison with the mice in the other two groups.”

24. “Moreover this effective protection was MUC1-specific, because the mice immunized with pcDNA3.1-VNTR could not prolong the survival time of the mice challenged with panc02 tumor cells (Figure4B)” is changed into “Moreover this effective protection was MUC1-specific, because the survival time of the mice immunized with pcDNA3.1-VNTR and challenged with panc02 tumor cells did not increase (Figure4B).”

25. “Splenocytes from immunized mice were isolated 7 days after the third time immunization, then co-cultured with panc02-MUC1 or panc02 cells at various E/T rations to measure the specific cytotoxicity activity” is changed into “To measure the specific cytotoxicity, splenocytes from immunized mice were isolated 7 days after the third time immunization, and then co-cultured with panc02-MUC1 or panc02 cells at various E/T rations.”

26. “These results confirmed the superior ability of pcDNA3.1-VNTR to induce MUC1-specific CTL than the other groups” is changed into “These results confirmed the superior ability of pcDNA3.1-VNTR to induce MUC1-specific CTL compared to the other groups.”

27. “These results showed the inhibitory effect of pcDNA3.1-VNTR vaccine on
tumor growth in vivo and also the anti-tumor activity induced by the pcDNA3.1-VNTR was specific for MUC1-expressing tumor cells” is changed into “These results demonstrated the inhibitory effect of pcDNA3.1-VNTR vaccine on tumor growth in vivo and indicated that the anti-tumor activity induced by the pcDNA3.1-VNTR was specific for MUC1-expressing tumor cells.”

28. “To investigate the protective and therapeutic effect of the DNA vaccine pcDNA3.1-VNTR against pancreatic cancer, we first constructed a murine pancreatic cancer cell which could express the human MUC1 protein and mainly on the cell membrane (Figure1 and Figure2). Although human MUC1 is homologous to murine MUC1, it is still a foreign protein and might induce an immune response from the host itself. In the preventive and therapeutic experiments, however, the differences between pcDNA3.1-VNTR, pcDNA3.1 and PBS groups challenged with panc02-MUC1 are large, as discussed below.” These sentences are added to the discussion section.

29. “In fact, our observations indicated that three intramuscle injections of pcDNA3.1-VNTR suppressed the development of pancreatic cancer expressing MUC1 in C57BL/6 mice and in a MUC1-specific manner” is changed into “Our observations indicated that three intramuscle injections of pcDNA3.1-VNTR suppressed, in a MUC1-specific manner, the development of pancreatic cancer expressing MUC1 in C57BL/6 mice”.

30. “Theoretically, any kind of vaccine finally should have the effect to cure the tumor” is changed into “theoretically, any kind of vaccine should be able to eradicate the tumor.”

31. “Their results showed that immunologic responses could be induced even in patients with advanced disease; however none of them achieved a long time survival” is changed into “Their results showed that immunologic responses could be induced even in patients with advanced disease; yet, long time survival could not be achieved.”

32. “Here we for the first time conducted the research on the pcDNA3.1-VNTR vaccine which had great therapeutic effect on the pancreatic cancer” is changed into “Our study was the first to use the pcDNA3.1-VNTR vaccine, which had a significant therapeutic effect on pancreatic cancer.”