Author's response to reviews

Title: Over-expressing Akt in T cells to resist tumor immunosuppression and increase anti-tumor activity

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Author's response to reviews: see over
Dear Editor,

Please find the revised manuscript titled “Over-expressing Akt in T cells to resist tumor immunosuppression and increase anti-tumor activity” that we submit for consideration at BMC Cancer.

Responses to Reviewer 1

Major compulsory revisions:

1. In the Background section, we explained in detail the importance of our study, why we engineer T cells to improve the efficacy of tumor immunotherapy (paragraph 1 and 2), and what are our major findings (paragraph 4). And in the Abstract, we also summarized the significance of our study. How to resist tumor immunosuppression and improve the efficacy of tumor specific T cells is a major problem for tumor immunotherapy, and our study provides a new strategy to increase the anti-tumor effects of adoptively transferred T cells. We believe our work will bring great attention from people in this field.

2. We used different experiments and models to examine if over-expressing Akt in tumor specific T cells can increase the activity and anti-tumor effects of T cells, and appropriate controls were used to substantiate our data. In Figure 1, to demonstrate Akt phosphorylation of T cells was inhibited by tumor, we used T cells cultured alone (Fig. 1A) or T cells isolated from tumor distant lymph
node (Fig. 1B) as control. In Figure 2, to demonstrate over-expressing Akt in T cells can improve T cell activity on exposure to tumor, we used T cells transduced with empty vector as negative control, and compared the proliferation and cytokine production of T cells with or without exposure to tumor. In Figure 3, to demonstrate over-expressing Akt in T cells can increase anti-tumor effects in vivo, we used T cells transduced with empty vector as negative control. To verify the above findings from mouse experiments, we used a human cancer model in Figure 5 and 6, all the experiments were designed carefully with necessary controls.

3. In the Discussion section, we summarized our major findings firstly (Line 271-273), discussed the results of our study and relevant literature, and then summarized the significance of our data in the Conclusions section.

Minor essential revisions

1. Background

According to the reviewer’s suggestion, recent studied have been addressed (Line 229, 242,244,318,326).

2. Methods

2.1 B16-OVA cell line was kindly provided by Dr. T.-C. Wu and Dr. Chien-Fu Hung’s lab in Johns Hopkins University (Baltimore, USA), which is mentioned in Acknowledgements. The cell line
was generated in their lab, and referred as reference number 12. The cells were cultured in RPMI1640 supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 2 mM non-essential amino acids, 50 units/ml penicillin and streptomycin, and the description of the culture conditions has been corrected in the Methods section (Line 107).

2.2 PLNCX empty vector was used as the control retrovirus, which has been added in Line 113.

3. Results

We have explained the reason why we performed each experiment in the first sentence, and summarized the data in the last sentence of every paragraph in the Results section.

4. Discussion

We have discussed the possible reasons that caused more apoptosis of myr-Akt transduced T cells than wt-Akt transduced T cells (Line 290-296), and provided a statistical analysis in supplementary Figure 3.

Reviewer 2

Major revision:

The recent report by Crompton et al found that inhibition of Akt enabled TIL a memory T cell signature with enhanced anti-tumor
effect. TILs isolated for adoptive transfer normally undergo extensive expansion process in vitro, and display a terminally-differentiated phenotype with short telomere and diminished anti-tumor activity. In their study, they added Akt inhibitor only in the expansion process, and found that TILs expanded with Akt inhibition persisted longer and exerted better anti-tumor effects. Comparing to their study, we used OT-1 T cells or PBLs stimulated and expanded in vitro, which displayed a CD62L+ phenotype after the short expansion process (supplementary Figure 2). Akt is necessary to induce and sustain effector functions of T cell [18], comparing to their study trying to enhance the persistence of T cells by inhibition of Akt, we tried to enhance the effector functions of T cells by over-expressing Akt, these two studies take different angles to manipulate T cells to enhance the anti-tumor efficacy, and are not contradictory to each other, and it’s worth further study to determine which means is superior to the other. In addition, in their work, tumor bearing mice were pretreated with total body irradiation, adjuvant vaccine, and IL-2 in conjunction with cell therapy, these preparations could ameliorate tumor immunosuppression and enhance the anti-tumor effects of cell therapy, however, severe adverse effects have been reported. Comparing to their study, we used adoptive cell therapy alone to
treat tumor, and over-expressing Akt could enable T cells to resist tumor immunosuppression, which is the major advantage of our strategy. These comments have been added in the Discussion section (Line 317-337) according to the reviewer’s suggestion.

Minor revisions

1. Subtitles have been added in the Results section.

2. We used protein L to determine the CAR transduction efficiency, and the data has been provided as supplementary Figure 1.

3. A graph showing the average and statistical analysis was provided in supplementary Figure 3.

We believe the quality of this paper has been improved, and it should generate broad interest in the field of cancer immunotherapy. Thank you very much for your attention and consideration.

Sincerely,

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