Peer Review File

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Reviewer Comments

The work by Qi T et al is interesting but I do not think it is suitable for publication at this moment. These are my main objections to the paper:

1. It seems to evaluate the efficacy of genome base-editing technology, however the title and some parts are focused in the antitumor activity of the inhibition of PD1. This may be confusing to the reader.

Answer: Thanks for your question. The PD-1/PD-L1 axis is an important signal pathway for tumor cells to escape during immunotherapy. Some studies have shown that the PD-1 gene knockout in T cells by CRISPR/Cas9 can improve treatment outcomes in patients. In our study, the adenine base editor (ABE)–xCas9 system was used to partially block the inhibitory signaling pathway of PD-1 in T cells and then observe its effect on tumor cells. Therefore, this study was mainly based on single gene editing technology to modify the PD-1 gene of T cells and then observe the control effect of T cells on tumor cells. The purpose of this paper is to provide readers with a PD-1 point mutation (PD-1-deficient T cells) that can achieve the same effect as pD-1 knockout, and we supplement some data to highlight this purpose(see Page 12, line 247-252).

2. It is surprising that no increase in cytokine production was found in PD-1 deficient T cells. Co-cultures of PD-L1 expressing A549 with either PD-1 deficient T cells or non-modified T cells might be an interesting experiment to support the role of PD-1 ITSM mutation. Is proliferation of these two groups comparable?

Answer: Thanks. No increase in cytokine production in PD-1 deficient T cells comparing to non-modified T cells confirmed the function of PD-1 deficient T cells was not be affected. The purpose of our experiment was to evaluate whether the function of gene-edited T cells was affected.
The aim of the co-culture experiment with A549 cells is to observe the killing ability of PD-1 deficient T cells to tumor cells, which is determined mainly by observing the reproduction of tumor cells after co-culture.

3. A more precise characterization of T cells would be required to confirm the results of both in vitro and in vivo results. Are the proportions of CD4 and CD8 T cells equivalent in all the groups? Are the stages of differentiation based on CD27 and CD28 expression?

Answer: Thanks. In our study, each T cell was obtained from the corresponding tumor model mouse, which was edited in vitro and then transplanted back into the mouse. So the proportions of CD4 and CD8 T cells are consistent with the proportion of each of the mice. This type of treatment is individualized.

4. Other methods to quantify cell lysis or apoptosis would support the results. At least annexin V/propidium staining would be required or, if available, real-time cell analysis.

Answer: Thanks for your advice. We add a cell apoptosis assay (see Page 7, 10-11, line 135-140, 210-213).

5. The text should be reviewed as some phrasing errors can be found (i.e. line 41 or line 48).

Answer: Thanks for your helps. The paragraph has been modified (see Page 3, line 44-48).