Peer Review File

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Reviewer A

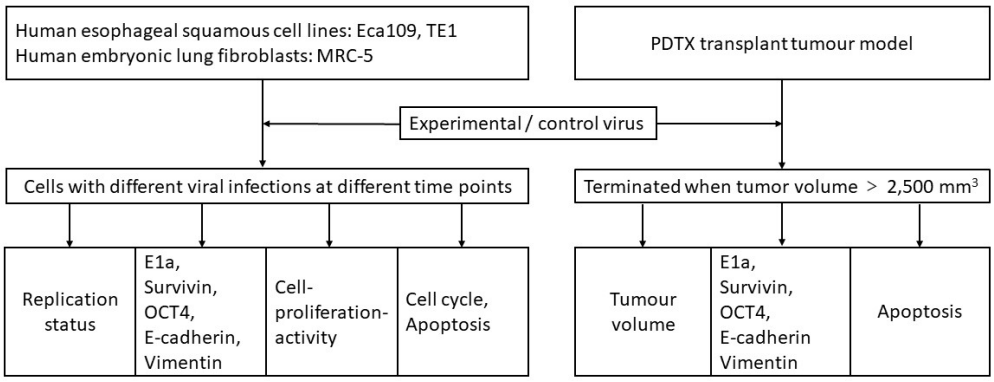
I would like to compliment Li et al on their manuscript titled "Oncolytic adenovirus-mediated dual knockdown of survivin and OCT4 improves therapeutic efficacy in esophageal cancer". In their experiment, they used an oncolytic adenovirus to successfully achieve knockdown of survivin an OCT4. Using this dual target design would have great potential in the treatment of esophageal cancer. The results look promising. I do have some comments:

(1) Please explain the sample size calculation more in detail.

Reply: Under the premise of meeting the 3R (Reduction, Replacement and Refinement) principles and statistics of experimental animals, a minimum of 6 mice per group is required.

(2) Perhaps the study protocol could be added as supplementary material.

Reply: A rough study protocol was submitted as supplementary material in the revised manuscript.



(3) In the discussion section: please make a recommendation for future research.

Reply: The part about our future research has been added in the revised cersion.

"In our future study, we will continue to explore the consistency and stability of anti-tumor effect of the oncolytic adenovirus. Besides, the underlying mechanism, especially the relevant signaling pathways, will be discussed and the side-effect of oncolytic adenovirus-mediated dual knockdown of survivin and OCT4 in vitro and in vivo will be explored."

(4) Although the results look promising, the study has several limitations. Please mention those limitations in the discussion section.

Reply: Limitations of our study has been listed in the revised manuscript.

"However, there are some limitations in our study. First of all, the replication ability of oncolytic adenovirus in vivo and the corresponding virus titer displaying anti-tumor effect were not discussed. Besides, the consistency and stability of anti-tumor effect of the oncolytic adenovirus need to be explored in the following study. At last, researches about the side-effects of oncolytic adenovirus-mediated therapy in vivo and in vitro were not presented."

Reviewer B

Excellent study.

(1) It would be good to do xenograft experiments with IV delivery, rather than intra tumor injection. But that can wait for the next series of experiments.

Reply: That was really a suggestion and we will explore and elucidate the above questions in a subsequent study.

Reviewer C

The manuscript was well written with good language and layout. It will be a valuable reference for the future esophageal cancer therapy.

I have only two questions.

(1) In line 89, the word 'alternately', just ask if you use the correct word for what you want to say.

Reply: We found “alternately” was inappropriate here and we have replaced it with “Meanwhile” to make the expression more precisely.

(2) Can you make and show the readers a diagrammatic illustration for the inhibition mechanism of knockdown of survivin and OCT4.

Reply: A diagrammatic illustration was added as an independent file in the revised manuscript.