Author's response to reviews

Title: A Novel Oncolytic Adenovirus Targeting Cyclin E Overexpression Repressed Lung Tumor Growth

Authors:

Pei-Hsin Cheng (paisin.paisin@gmail.com)
Xiao-Mei Rao (x0rao001@exchange.louisville.edu)
Stephen L Wechman (slwech01@exchange.louisville.edu)
Xiao-Feng Li (x0li0027@exchange.louisville.edu)
Kelly M. McMasters (kmmcma01@exchange.louisville.edu)
Heshan Sam Zhou (heshan.zhou@louisville.edu)

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

I would like to thank you for organizing the prompt review of our manuscript entitled “A Novel Oncolytic Adenovirus Targeting Cyclin E Overexpression Repressed Lung Tumor Growth”. We have addressed all the comments from the reviewers and highlighted the major changes in the revised manuscript. Provided below is our point-by-point description.

Reviewer #1

**Question (Q) 1:** “...ED-1 cells containing a human cyclin E gene under the control of the SPC promoter...To restore SPC promoter activity, cells need to be grown in 3D culture or reintroduced into animals.” **Response (Res):** We thank reviewer for pointing out this information, we already added this in the Methods and Results sections to highlight the importance of our animal study (page 7, line 115-116 and page 14, line 272-278).

**Q2:** “In the supplemental figure S1 while they could detect some human cyclin E expression through PCR...How do they know it isn’t murine cyclin E protein.” **Res:** The PCR was performed with primers specifically targeting human CCNE2 gene. We already added the primer information in the figure legend (page 31, line 690-692).

**Q3:** “It would help if the authors could clearly explain why overexpressed human cyclin E in these cells would make them more permissive to oncolytic adenovirus replication...” **Res:** We have added the explanation in Discussion section per reviewer’s request (page 17, line 333-340).

**Q4:** “The murine tumors had an unusual phenotype in that hexon viral protein encapsulated cells isolating them from adjacent cells and also isolating the virus. Has something like this been seen before and if not does this impact the enthusiasm for this model.” **Res:** In a previous clinical study, oncolytic Ads were observed in clusters of 5-20 cells after intratumoral administration, indicating that Ad spread in tumors is restricted (Galanis, et al. Gene therapy 2005, 12(5):437-445). Considering the difficulty in obtaining clinical samples for studies, such a preclinical system as ED-1 model will benefit the development of the strategies to enhance viral penetration and spread within solid tumors. We have added this information in the Discussion section (page 18, line 362-366, 373-376).

**Q5:** “Figure 5 has panels A-C and then panel B has a-d. This gets a little confusing. Make a-d panels, 1-4 or i-iv.” **Res:** We accepted reviewer’s suggestion and made the change to i-iv.

Reviewer #2

**Q1:** Some reports have indicated that Onyx-015 is only permissive in p53-deficient cells. In the manuscript, Onyx-015 is permissive in a wild type p53 A549 cell line. Can authors explain this discrepancy? **Res:** E1b55K-deleted Ads was proposed only able to replicate in p53-deficient tumor cells, as the E1B55K-mediated degradation of p53
protein was not required in those cancer cells. However, the original hypothesis was challenged by several studies showing that E1b55K-deleted Ads are able to replicate in cells regardless of their p53 status. We have demonstrated that cyclin E dysregulation or overexpression in cancer cells is an important molecular basis of selective replication of E1b55K-deleted Ads in human cancer cells. The information has been added in the Introduction section (page 5, line 68-72).

Q2: Is Ad-cycE similar to Onyx-015 with the E1B-55K deletion? Can authors elaborate on this virus' replication selectivity? **Res:** With the deletion of entire E1b region, Ad-cycE shares the replication pattern similar to E1b55K-deleted dl1520 (Onyx-015) which relies on the cyclin E overexpression in cancer cells. The further information of the Ad-cycE has been added in the Introduction section (page 6, line 93-100).

Reviewer #3

To better address the questions from Reviewer #3, we organized the related descriptions into several major points.

Q1: “… conclusions about how the immune system may influence oncolytic viral replication and/or therapeutic efficacy (included in the Discussion) cannot be drawn without at least initial analysis of immune cell infiltration into tumors across treatment groups…” “the manuscript would be significantly strengthened by some analysis of immune cell infiltration into the tumors in the presence/absence of virus…” **Res:** Thanks for reviewer’s suggestion. To satisfy this concern, we have changed the discussion and pointed out the importance of immune study for the future direction (page 18, line 355-357). We agreed with reviewer that the manuscript could be significantly strengthened by adding the experiment. However this report does not intend to cover the study of the immune effects on virotherapy. This work focused on Ad replication in murine cells and evaluated the Ad repression of mouse tumor growth in vivo. Considering the important suggestion from reviewer, our ongoing step is to study the influence of immune-mediated effects on the antitumor efficacy of the oncolytic virus with ED-1 model.

Q2: “…confirming the cyclin E2-CDK2-Rb mechanism of viral replication) are missing and would strengthen the story if included…” “…showing alterations in cyclin E-CDK2-Rb signaling between treatment groups would validate their proposed mechanism…” **Res:** We already published the work that demonstrated the role of cyclin E-CDK2-Rb mechanism in oncolytic Ad replication in PLoS One 2013, 8(2):e57340. As we mentioned above, this work focused on evaluating the potential of ED1 model in the study of oncolytic virotherapy. Though this is beyond the scope of current study, we agreed that such a model with the unique background of cyclin E overexpression can provide a suitable in vivo environment for researchers to further study details of cyclin E-CDK2-Rb mechanism in oncolytic adenoviral replication and have added this information in Conclusion section (page 19, line 380-382).

Q3: “…looking at growth and therapeutic efficacy in the context of the lung microenvironment (e.g. orthotopic allograft) would add more clinical relevance to their study…” **Res:** We agreed reviewer’s point and added this in the Conclusion section to illustrate the future direction (page 19, line 386-389).
Q4: Bright field photomicrographs presented in Figure 2 were of poor quality/too small and therefore difficult to interpret. **Res:** We have improved this by enlarging the figures to the limit of page.

Q5: “… while the authors speculate about the nature of the capsule structures formed around infected cells within the tumor…Are they similar to what is observed in human tumors treated with oncolytic virus? Have those components been identified…” **Res:** This question is similar to the question 4 from Reviewer #1 and has been addressed above.

Q6: “…The manuscript is well-written and clear, but could benefit from including a rationale for some of their studies…” **Res:** We accepted the comment and made changes in page 11, line 200-201 and 211-212; page 12, line 233-235; and page 14, line 272-278.

We appreciate that BMC cancer offers us a chance for the resubmission and hope the revised manuscript is more acceptable now.

Sincerely yours,

H. Sam Zhou, Ph.D.
Associate Professor
Departments of Microbiology and Surgery
J. Graham Brown Cancer Center
University of Louisville Health Sciences Center
Tel: 502-852-5745
Fax: 502-852-2356