Author’s response to reviews

Title: Oncolytic Targeting of Androgen-sensitive Prostate Tumor by the Respiratory Syncytial Virus (RSV): Consequences of Deficient Interferon-dependent Anti-viral Defense

Authors:

Ibtissam Echchgadda (echchgadda@uthscsa.edu)
Te-Hung Chang (changt@uthscsa.edu)
Ahmed Sabbah (sabbah@livemail.uthscsa.edu)
Imad Bakri (bakri@uthscsa.edu)
Yuji Ikeno (ikeno@uthscsa.edu)
Gene B Hubbard (hubbardg@uthscsa.edu)
Bandana Chatterjee (chatterjee@uthscsa.edu)
Santanu Bose (bose@uthscsa.edu)

Version: 3 Date: 18 November 2010

Author’s response to reviews: see over
Reviewer comments

Reviewer # 1

All comments of Reviewer # 1 were addressed satisfactorily in the last submission. Reviewer # 1 does not have additional comments.

Reviewer # 2 - comment # 1

Comment #1: It would be strongly recommended to investigate the impact of RSV without androgen on the growth of LNCaP cells both in vitro and in vivo.

Response: In new experiments we show that the viral replication for RSV is more efficient in androgen-dependent LNCaP cells in the presence of R1881, a non-metabolizable synthetic androgen (Figure 2 a-c). LNCaP cells were grown in androgen-depleted (charcoal stripped media) and then either ethanol (vehicle) or R1881a (1 nM) was added to the media for overnight before mock infection or infection with RSV was performed. The RSV titer was ~2.2-fold higher with androgen treatment of LNCaP cells compared to vehicle treatment (Figure 2a). The higher RSV titer in the presence of androgen caused more extensive apoptosis of LNCaP cells in the presence of androgen (80% apoptosis) than in the absence of the hormone (50% apoptosis) (Figure 2c). Given the androgen dependence of these cells, the much higher apoptosis of mock-infected cells in the absence of androgen is expected.

Experiments were also carried out with the C4-2B androgen-independent prostate cancer cells (a derivative of LNCaP cells, androgen receptor positive). To assess the effect of androgen on RSV-induced oncolysis, the cells were cultured in charcoal stripped media, incubated overnight with vehicle (ethanol) or R1881 (1 nM) and then were infected with RSV. RSV replicated efficiently in C4-2B cells, and the virus titer was higher in androgen-treated cells than in vehicle-treated cells (Figure 2d). Androgen was not required for the RSV-induced oncolysis of C4-2B cells (Figure 2e).

Due to the 3-week timeline given to us for submission of a revised manuscript, we could not carry out in vivo studies to assess the effect of androgen in RSV-induced oncolysis of castration recurrent xenograft tumors. The editor’s letter from the BMC-Cancer (sent on October 28) indicated that the manuscript revision is due by November 18. It takes ~8 weeks to grow LNCaP xenograft tumors in testes-intact nude mice. A similar time frame also applies to the growth of androgen-independent xenograft tumors from C4-2B cells. The re-emergence of castration resistant tumors from LNCaP cells requires additional many weeks. Thus it will take several months before we are able to determine the effect of androgen in vivo on the RSV-induced oncolysis of xenograft prostate tumors that express the androgen receptor.

We hope that the reviewer and editor will appreciate this difficulty in the timeline for in vivo experiments and that the additional data in Figure 2 would satisfy the concern of the Reviewer # 2. Please note it has been more than eight months since our original manuscript was submitted to BMC Cancer.

Reviewer # 2 other comments that were addressed satisfactorily in the previous submission

Comment #2 – “The authors examined the effect of RSV through the intratumoral injection. However, if RSV would be applied to clinical practice, it might be much more variable to administer it systemically targeting metastatic diseases. Accordingly, the authors should give comments on this issue”.

Response – We previously addressed this comment and modified our discussion, as asked by the reviewer. The paragraph below is a repeat of our previous response.

Echchgadda et al
We are aware of the importance of the route of RSV delivery in order to fulfill the clinical potential of RSV therapy. In an earlier report (Echchgadda et al, Cancer Gene Ther. 16:923-935, 2009), we have demonstrated that intraperitoneal (i.p.) injections of RSV are also effective in causing regression of PC-3 xenograft tumors which are more aggressive than LNCaP xenograft tumors with regard to tumor growth. We are currently developing microencapsulated RSV preparations for delivery to tumor-bearing mice through intravenous (i.v.) injections or oral gavage (p.o.). These issues are now described in the “discussion” section of the revised manuscript.

**(Minor Essential Revisions):** Please note that all comments were addressed in our previous responses. We are repeating our responses as below.

1. Comment – “The last sentence in Results of Abstract section should be omitted or moved to the Conclusions”.
   Response – The sentence has been deleted.

2. Comment: The last paragraph of Background section overlaps Results section.
   Response – The last paragraph has been removed.

3. Comment: The description regarding the characters of RM1 cells in the first paragraph of Results section should be moved to Methods section.
   Response: In the revised manuscript, RM1 cells are described in the “methods” section.

4. Comment: The last two paragraphs of Results section should be omitted or moved to Discussion section.
   Response: We have reorganized the last two paragraphs of the Results section and a revised version of these paragraphs is now part of Discussion.

5. Comment: The last sentence of the third paragraph of Discussion section seems to be evidently overdiscussed.
   Response: We have revised this paragraph in the “discussion” section.

6. Comment: There is no description concerning the guideline of animal experiments followed by the authors.
   Response: This is now included in the “Methods” section.