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

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Reviewer-1

1) Comment – “My major concern is that the authors did not show viral replication in tumor mass after intratumoral injection in animal models. If the virus is replicating in the tumor, why repeated injections were needed? This is an important point since it distinguishes whether the tumor regression is caused by true viral lytic infection and dissemination or by viral induced apoptosis but also self-elimination in the tumor. If the authors are not able to answer this question at this time, at least they need to discuss this point in the Discussion”.

Response – This is a very valid point. In new data we show that the homogenate from the RSV-injected tumor mass has very high viral titer (Fig. 3c), while as expected, the medium-injected tumor mass showed no trace of RSV. This indicates that the virus indeed replicate and thus possibly enters into a lytic cycle within the tumor cells which in turn lead to the tumor cell ablation through virus-induced apoptosis and cell lysis. Due to the copious virus amplification in the tumor cells, repeated injections may not be necessary in order to induce tumor regression. However, we have not checked this possibility yet and we plan to examine this in future work. This possibility has been discussed in the revised manuscript. In addition, we failed to detect Th1 (IL-10) and Th2 (IFN-#) cytokines in the tumor micro-environment of tumors injected with RSV (Fig. 5b). Therefore, our results argue against self-elimination of tumors as the cause of tumor regression following RSV administration.

2) Comment – “The tumor growth was only observed up to 14 days after the last viral injection. Have authors observed longer time period after viral treatment stopped?”
Response - We added new results (Fig. 5a) to address this point. We show that in deed long-term tumor regression could be maintained after RSV injection. We failed to detect tumor reoccurrence at 44d following the last RSV injection (Fig. 5a).

3) Comment – “Initial tumor size when the virus was injected should be indicated.”

Response - Initial tumor sizes varied in individual animals. For example, before the start of the injections, LNCaP xenograft tumor volumes ranged from 100 to 300 mm³, and for RM1 tumors the volumes ranged from 56 to 164 mm³ (RM1 tumors are highly aggressive and thus we started injections at lower tumor volumes). That is why, tumor growth kinetics were plotted by normalizing each data point against the corresponding tumor volume at day 1 (the starting point for RSV/medium injection), which was set as 100%. We have now provided the range of tumor volumes for each prostate cancer cell-line (pg-6, line-21 and pg-7, line-11).

4) Comment – “In RM1 syngeneic model, do we know if antibody against RSV is generated? How long it takes the animal to generate the antibody and would that affect the efficacy of the repeated viral injection?”

Response - We have not checked this point yet and plan to do so in future work. However, in immune-competent C57BL/J mice, RSV administration (via i.p.) elicited very weak humoral Th2 adaptive-immune response (as measured by examining levels of Th2 cytokine IL-10 in the spleen) (Fig. 5b). Since Th2 response is critical for antibody generation, we speculate that antibody response against RSV will be minimal. In addition, we have provided new data to demonstrate that repeated RSV injection is not required to support long-term tumor regression (Fig. 5a); since tumor did not reappear even at 44d following last RSV injection.

5) Comment - Fig. 4 should indicate the time point when the animals were killed. Also, Why TUNEL staining shown in Figure 4a seems not a usual nuclear staining Pattern.

Response – For Fig. 4 (Fig. 4b and 4c in the current revised manuscript) the RM1 tumors were derived 12h after two RSV injections (2d apart) via intratumoral route. In contrast, LNCaP tumors were derived 12h after three RSV injections (2d apart) via intratumoral route. This information is now included in the figure legend.

At this time we are not sure why the TUNEL staining for LNCaP tumors lack prominent nuclear staining. We speculate that diffuse staining may reflect cleaved DNA derived from cell-free aggregated nuclear debris from dead cells.

Reviewer - 2
1) Comment – “The significance of this study is almost similar to that of previously published manuscript by authors same as this study. Although authors emphasized that they used androgen-dependent LNCaP cells in this study, they had not assessed the effect of RSV on LNCaP cells considering their androgen-dependent characteristics. It would be strongly recommended to investigate impact of RSV without androgen on the growth of LNCaP cells in vitro and in vivo”.

Response – This is an excellent point and it is the subject of our rigorous scrutiny in future experiments. We will determine the effect of RSV in vivo on xenograft tumors that are androgen-independent for growth despite expressing androgen receptor (AR). Castrated mice will be used to produce androgen-independent, AR expressing xenograft tumors. Future studies will also include determining the effect of RSV on LNCaP cells grown in the absence of androgen in charcoal stripped serum. The interrelationship between the oncoytic activity of RSV and androgen in the context of prostate cancer cells is an intriguing issue which we will address in a separate study and thus is not included in the present study.

2) Comment – “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 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 (pg-16, line-19).

3) Comment – “The last sentence in Results of Abstract section should be omitted or moved to the Conclusions”.

Response – The sentence has been deleted.

4) Comment – “The last paragraph of Background section overlaps Results section”.

Response – The last paragraph has been removed.

5) Comment – “The description regarding the characters of RM1 cells in the first paragraph of Results section should be moved to Methods section”.

Response – The characters of RM1 cells are now described in the Methods section.
Response – In the revised manuscript, RM1 cells are described in the “methods” section (pg-5, line-17).

6) 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.

7) Comment – “The last sentence of the third paragraph of Discussion section seems to be evidently over discussed”.
Response – We have revised this paragraph in the “discussion” section.

8) Comment – “There is no description concerning the guideline of animal experiments”.
Response – This is now included in the “Methods” section (pg-7, line-15).