Author's response to reviews

Title: Neural protein gamma-synuclein interacting with androgen receptor promotes human prostate cancer progression

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Author's response to reviews: see over
Dear Dr. Chap

Thank you very much for your letter of July 10, 2012, concerning our manuscript entitled “Neural protein gamma-synuclein interacting with androgen receptor promotes human prostate cancer progression” (manuscript number: 1116962922695619).

We appreciate the reviewers’ comments and have revised our manuscript accordingly. For point-by-point responses to the reviewers’ comments, please see the letter to the reviewers on the subsequent pages. We believe that the revised manuscript is now ready for your further consideration for publication in BMC Cancer. Please do not hesitate to contact us if you have any problems.

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

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Comments from Prof Labrie:

I have reviewed the authors’ reply to my previous comments. Unfortunately, many of these still stand.

1. The authors replied that they have demonstrated the efficacy of the siRNA (Fig.1B) and that they also confirmed SNCG overexpression in stably transfected LNCaP-AI cells (Fig. 4A) as well as overexpression/knock-down of SNCG in stably transfected LNCaP cells (supplementary Fig. 6). However: (1) they do not provide evidence of SNCG overexpression in LNCaP cells that were transiently transfected and harvested at different intervals up to 7 days (Fig. 1), and (2) while the siRNA is effective 72 hours post-transfection (Fig. 1B), some experiments with siRNA lasted as little as 20 hours (Fig. 2) or as long as 7 days (Fig. 1C). Thus, important controls are still lacking.

Thank you for your comments. We have confirmed that SNCG protein expression in LNCaP cells, which were transiently transfected with SNCG plasmid or siRNA, was increased or decreased at different intervals up to 7 days (Suppl. Fig. 7). For Figure 2, we are sorry that we did not describe the experimental method clearly. After transfection for 24 h, the cells were collected in an upper Transwell chamber in which the cells had previously been incubated for 20 h for cell migration assays, and 48 h for cell invasion assays. We have added to the description in the “Cell migration assay” and “Cell invasion assay” parts of the Materials and Methods section.

2. The authors replied that they added this information in the Materials and Methods but it should be mentioned in the appropriate sections of the Results and corresponding figure legends. If the authors need to use a term such as "cytological", please consider using "cell culture" instead.

We added the description of “transient transfection” in the corresponding sections of
results, methods and figure legends (P7,8,9,10,13,14,33). On P7, we corrected “cytological” to “cell culture”.

3. The authors replied that they used a pool of stably transfected cells but the manuscript says “after selection by puromycin treatment, a RFP positive clone was selected for utilization in the subsequent experiments”. Were the experiments performed using a single clone, a pool of transfected cells, or multiple independent clones?

The experiments were performed using multiple independent clones.

4. The information added by the authors is useful but it is not sufficiently precise (“higher”, “stronger”, “much higher”). Please be more quantitative and/or refer the reader to “solid” data. In addition, this information should be provided in the Results section when LNCaP-AI cells are first mentioned, not in Materials and Methods.

We have added the comparison of AR mRNA expression between LNCaP and LNCaP-AI cells to Supplementary Figure 4. The data for LNCaP-AI cells were moved to the first part of the Results section.

5. The author’s reply is acceptable.

6. The figure legends still do not meet current scientific standards and, contrary to the authors’ reply, most of these appear unchanged compared to the previous version. Some of the more striking examples are the legends to Fig. 3D-F and Fig. 4B-C.

Thank you for your attention, we have modified the figure legends in the revised manuscript.

7. The authors have added important information to the manuscript but the “tumour
imaging” is still not explained. In addition, there are no “tumour imaging” data in the manuscript. Please delete all references to “tumour imaging”.

In addition, please explain why some Western blots are presented as black bands on a white background (Fig. 1A) whereas others are presented as white bands on a dark background (Fig. 1B).

This was our mistake: we wanted to present the “mice photo” and/or “tumor size”. We have deleted the “tumor imaging” data (P18). Our lab has three western blot imaging systems, in which the imaging conditions are different.

8. Answer accepted.

9. I understand and accept the authors’ reply. However, ideally they should have varied only one parameter i.e. by either overexpressing or knocking down SNCG in both intact and castrated mice. Instead they changed two parameters simultaneously by comparing SNCG knockdown in intact mice to SNCG overexpression in castrated mice.

Thank you for your advice; we will pay attention to these details in our future work.

10. The author’s reply is acceptable.