Reviewer’s report

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

Version: 3 Date: 20 June 2012

Reviewer: Claude Labrie

Reviewer’s report:

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

Major Compulsory Revisions

1 – The majority of the experiments rely on SNCG overexpression or knock-down but several of these experiments lack an important control: the authors should provide data showing SNCG protein levels for these experiments. The data should allow the reader to compare SNCG levels in control and over/underexpressing cells. This concerns the following figures: 1C-D, 2A-B, 3C-F and 5A-F.

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.

2 – In relation to the previous comment, the text of the paper does not always explicitly state if the experiments were performed using stably or transiently transfected cells. Please make this clear.

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.

3 – The authors used a lentiviral vector to generate stable cell lines. They should specify if the experiments were performed using a pool of stably transfected cells or individual clones.

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?

4 – The authors performed several experiments in LNCaP-AI cells. They should state how these cells compare to LNCaP cells in terms of AR levels, response to androgens, proliferation, etc.

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.

5 – The AR-SNCG coimmunoprecipitation experiments were performed using LNCaP cells that stably overexpress SNCG. However, SNCG seems to be quite abundant in untransfected LNCaP cells. The authors should state if they attempted to co-immunoprecipitate AR and SNCG in untransfected LNCaP cells. If so, did they observe an interaction between the two proteins?

The author’s reply is acceptable.

6 – The figure legends need to be completely rewritten for two reasons. First, they generally do not contain enough pertinent information to enable the reader to understand the figure/experiment. Second, most of the figure legends contain statements that interpret the data. With the exception of general figure titles, these statements should be removed.

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.

7 – Experimental details are lacking for several experiments including the transwell chamber assays and the quantitative RT-PCR assays. In the Methods section the authors do not state which antibodies were used to immunoprecipitate AR and SNCG. In reference to the nude mouse experiments the authors mention “tumour imaging”. What is this exactly? Please ensure Methods are sufficiently detailed.

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).

8 – In reference to the experiment presented in Fig. 5A-C, the authors state that “a significant delay” in tumour growth was observed in siRNA-producing tumours compared to controls. This is surprising given the overlap in the error bars. Do
they stand by their interpretation of the data?

Answer accepted.

9 – I do not fully understand the rationale for evaluating the effect of SNCG overexpression on LNCaP tumour growth in castrated male mice (Fig. 5D-F). In reference to this experiment the authors state that “there is no significant difference between two groups with different expression levels of SNCG, indicating that SNCG regulates androgen-dependent prostate tumorigenesis.” I am not certain that this experiment was designed to address this question. Please clarify.

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.

10 – The authors should define what they consider to be “androgen-independent” tumours (Ref. Table 1 and Fig. 6). Are these relapsing tumours?

The authors’ reply is acceptable.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests