Reviewer’s report

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

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Reviewer: Claude Labrie

Reviewer’s report:

Gamma synuclein is a protein that has been implicated in cancers of various cellular origins and whose molecular mechanism of action appears complex. In this paper Chen et al. investigated the contribution of gamma-synuclein (SNCG) to androgen-regulated prostate cancer cell proliferation. They report that SNCG is more abundant in androgen-sensitive LNCaP prostate cancer cells than in androgen-insensitive cell lines (Fig. 1A) and that androgens up-regulate SNCG expression (Figs. 3A,B). Overexpression of SNCG in LNCaP cells accelerated cell proliferation in vitro whereas down-regulation by siRNA had the opposite effect (Fig. 1C). The authors show that AR and SNCG can be co-immunoprecipitated from LNCaP cells (Fig. 3C) and that SNCG knock-down by siRNA inhibits DHT-induced PSA transcription and ARE reporter gene activity (Figs. 3D,F). Conversely, SNCG overexpression in androgen-independent LNCaP-AI cells generally enhanced androgen-dependent transcription and proliferation (Fig. 4). siRNA-induced knock-down of SNCG caused LNCaP cells to accumulate in G1 (Fig. 1D), inhibited LNCaP invasion in transwell assays (Fig. 2) and slightly reduced LNCaP tumour growth in nude mice (Fig. 5A-C). Finally, analysis of SNCG levels by immunohistochemistry in prostate tumour specimens confirmed that SNCG overexpression is associated with prostate cancer (Fig. 6 and Table 1).

Comments

This is an interesting paper in which the authors used complementary approaches (overexpression versus siRNA, LNCaP vs LNCaP-AI cells) to examine the role of SNCG in androgen-dependent proliferation of prostate cancer cells. The experiments appear to have been well controlled in general and it is stated that the data are reproducible (n=3). Depending on the authors’ responses to the specific comments below, I would say that the data presented in this paper support the claims that SNCG is overexpressed in prostate cancer, that it participates in androgen-regulated gene expression and that it probably contributes to cancer cell proliferation. This report is novel and should generate considerable interest regarding the roles of SNCG in prostate cancer and androgen action.

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.

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.

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.

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.

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?

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.

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.

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 the authors stand by their interpretation of the data?

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

Minor Essential Revisions

11 – There are a few typographical/spelling errors such as “SCNG”, “Ablate-Shen”. Please revise.

12 – If appropriate, please correct the y-axis labels of Figs. 5C and E to read “Mean Tumor Volume (cm3)”.

13 – The manuscript is generally well written and while sentence structure could be improved in some areas, the language is generally acceptable.

Discretionary Revisions

14 – Did the authors examine the effect of SNCG overexpression on invasion?

15 – The Introduction could be shortened by removing the first paragraph that discusses the management of prostate cancer patients.

16 – The discussion is lengthy and could probably be shortened to avoid repeating what is already stated in the Results section. On the other hand, the authors should consider discussing the possible mechanism(s) whereby SNCG enhances AR activity.

**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