Reviewer’s report

Title: APC2 is Critical for Ovarian WNT Signalling Control, Fertility and Tumour Suppression

Version: 0 Date: 14 May 2019

Reviewer: Reviewer 2

Reviewer's report:

PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?
Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?
Yes - the approach is appropriate

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?
Yes - experiments and analyses were performed appropriately

STATISTICS - Is the use of statistics in the manuscript appropriate?
Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?
Yes - the author's interpretation is reasonable

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?
Yes - current version is technically sound

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: The manuscript investigates the functional role of APC2 in ovarian homeostasis, fertility and the development of granulosa cell tumors (GCT) in mice. It is demonstrated that APC2 supports fertility, with its deficiency causing intra-ovarian defects and leading to fertility problems. Loss of APC2 induces WNT signaling in ovaries as well as Foxo1 expression which causes apoptosis in APC2-/- follicles. The paper also indicates an involvement of the PTEN regulated PI3 kinase/AKT pathway with a decreased activity observed in the APC2-/- setting. Further, the absence of APC2 in ovaries results in impaired vascularization and steroid genesis and - most importantly -
predisposes for GCT.

Overall, the authors provide convincing experimental evidence to substantiate their conclusions. The appropriate experiments were carried out and they were interpreted in a correct way. This manuscript presents new and detailed insights on the significance of APC2 in the context of the ovaries.

ADDITIONAL REQUESTS/SUGGESTIONS:

Major comments:
1. A very interesting outcome of this study is the fact that APC2 deficiency appears to facilitate tumorigenesis as judged from the development of granulosa cell tumors (GCT). It is shown that tumor markers commonly observed in human GCT were also aberrantly expressed in the GCT observed in the murine models. These observation lead to the statement that the authors generated a pre-clinical model for GCT. Conversely, are there indications that in human GCT APC2 is mutated or deficient? This is something that should be discussed.

2. Note that in the PDF that was generated for review purposes the resolution of the Figures is mediocre at best. Please make sure high-resolution figures are available.

3. The conclusion section of the abstract is rather limited as it focuses on the observed GCT formation only. Please rephrase to include the fertility effects as well.

Minor comments:
1. Check spelling of "gonadotropin / gonadotrophin" and be consistent throughout the manuscript.
2. Page 4, lines 90/91 - "gain of function mutation" should be "gain-of-function mutation".
3. Page 5, line 116 - What exactly is the mixed background of the mouse models? Please mention this in the Methods section.
4. Page 8, line 202 - The reference to "Figure 2d, e & g" is not appropriate at this point. Here, should be referred to Figure 1g.
5. Page 16, line 421-436 - Please adhere to gene annotation rules and do not use capitals when murine genes/proteins are indicated.
6. Page 32, line 856-857 - The caption of Supplementary Figure 4 given here does not fully correspond to the caption given in the Supplementary figure legends file.
7. Figure 3c - Please indicate in the figure what the cells are stained for, making this figure self-explanatory.
8. Figure 7 - Indicate in or below the micrographs for which antigen was stained. This again makes the figure easier to read.

Note: This reviewer report can be downloaded - see attached pdf file.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes
**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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