Reviewer's report

Title: The oncogenic effects of HES1 on salivary adenoid cystic carcinoma cell growth and metastasis

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Reviewer: Vladislav Korobeynikov

Reviewer's report:

In this article the authors describe a role of HES1 in regulating proliferation, migration and apoptosis in salivary adenoid cystic carcinoma (SACC). In general, the manuscript is well written but it raises several concerns that should be addressed prior to publication.

Major concerns:

1. The authors do not make it clear what cell lines for which experiments they used. The Materials and Methods section includes two SACC cell lines, however, the figures apparently show the sets of experiments from only one cell line. Could you please specify which cell lines was used for which sets of experiments? In addition, in order to make any statements about the mechanism a full set of experiments in at least two independent cell lines is required.

2. Elevated levels of full length caspases (figure 3F) does not necessarily mean an increase in apoptosis. The authors should provide a Western blot image with bands for cleaved species of caspases 3 and 9 and report a ratio of cleaved to full-length forms.

3. If the authors speculate that HES1 affects cell growth they should provide a data of cell-cycle assay or similar method showing different distribution of cell cycle phases in cells upon HES1 knockdown.

4. The authors used siRNA knockdown for xenografts study with an endpoint at 18 days after inoculation. siRNA knockdown is not stable and lasts on average for around 5-7 days depending on the cell type. Taking this in consideration, the authors should provide IHC images of tumors stained for HES1 and/or Western blot from tumor lysate to compare the levels of HES1 at the endpoint.

5. In the sentence in page 16 line 2 the authors state “…reductions in HES1 expression in SACC cells could induce cellular apoptosis to inhibit cell growth.” This is misleading.
Induction of apoptosis will result in inhibition of tumor growth, not necessarily cell growth (proliferation). If the authors state that HES1 knockdown also affects cell proliferation in vivo they should provide a confirmation to this hypothesis such as IHC images of tumors stained for Ki67 and calculate proliferative index.

6. The authors' data suggests that HES1 knockdown affects cell migration. Could that be observed in vivo? Do the mice injected with HES1 knockdown cells develop less metastatic lesions than the mice injected with the control cells?

Minor concerns:

1. The article contains orthographic and grammatical errors that, however, do not significantly affect the clarity of the text. Several examples are provided below.

   a. Page 6 line 1 "...pathway that could active HES1."

   b. Page 13 lines 10-13 contain a confusing sentence.

   c. The sentence on page 18 line 13 is confusing.

2. Table 1 is a bit misleading - it contains two sets of sequences per single siRNA. Could you please clarify what exactly what used in the study?

3. Some links to the figures in the text seem to be incorrect. For example, page 13 line 10 has a link to figure 2I although it apparently should be 1I, page 14 line 10 should have a link to figure 2B instead of 2A.

4. All Western blot images lack indication of molecular weights of the bands and quantification.
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.

No

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

No

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