Reviewer's report

Title: Apoptosis inhibitor-5 overexpression is associated with tumor progression and poor prognosis in patients with cervical cancer

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Reviewer: Pavel Krejci

Reviewer's report:

This study reports the API5 effect on cell growth in vitro as well as its in vivo expression and prognostic value in a relatively large collection of cervical cancer specimens. Overall the study appears sound and brings important information about API5 and its potential role in cancer. I have several points which need to be addressed before the article is accepted for publication.

1. Background: I do not think there is enough evidence identifying API5 as key molecule in tumor progression.

2. Fig. 1C: A phase contrast image showing cytoplasm should be included. More cells should be shown including those with cytoplasmic API5 staining as mentioned in text. The endogenous API5 signal in CaSki cells should also be demonstrated by ICC figure.

3. In Fig. 2A it seems that only the upper form of API5 is downregulated following the siRNA treatment. This should be discussed because the authors consider both bands to be API5. Forced API5 expression leads to upregulation of both bands in 293T cells whereas only upper band is upregulated in CaSki cells. Also the increase of API5 signal in 293T cells is only marginal despite the fact that these cells usually express high levels of transgenic proteins after routine transfection. Authors should provide more data in order to clarify these discrepancies.

4. Fig. 2B, C: The data on B imply that majority of 293T and CaSki cells were transfected. No information is given regarding the transfection efficiency. C, showing pictures of stained cell colonies would strengthen the results.

5. Methods: Authors state that tissue culture media in colony formation assay was changed every 7 days. This does not look like a proper cultivation practice. Why was not the media replaced every 2-3 days as usual?

6. Fig. 2A: No time frame is mentioned for the RNAi experiment done in HeLa cells. How long the RNAi-mediated API5 downregulation persisted in cells, considering that the colony forming assay took 2 weeks.

7. Sample numbers stated in abstract should reflect the numbers actually interpreted in the study, not total numbers.


9. Lines 338 and 342: Colonogenicity does not appear to be the right term here.

10. Authors should provide stronger link between API5 and ERK activation, since
it is not clear why the clinical section of the paper analyzes ERK activation together with API5 expression. I wonder if the ERK activation increased/decreased in cultured cells along with modulation of API5 levels by overexpression and RNAi. Also, the discussion lacks comparison of ERK expression data obtained here with those already published. I assume this is not the first study addressing the ERK expression in cervical carcinoma.

11. Legend to Fig. 1B: Is Lamin B a H2B, please clarify.

12. Legend to Fig. 2C: is the data shown an average from triplicate in one experiment, or from three independent experiments? Please clarify.

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