Reviewer's report

Title: LATS2 is De-methylated and Overexpressed in Nasopharyngeal Carcinoma and Predicts Poor Prognosis of the Patients

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Reviewer: Hiroshi Nojima

Reviewer's report:

In this manuscript, Zhang et al. responded properly to several comments, but appears to have ignored some of the essential criticisms. At least, the following 2 criticisms should not be neglected.

Major concerns:

Criticism #2:
In terms of Lats2 function in cancer cells, the authors should not ignore the contribution of the Hippo pathway to the regulation of tumor growth. It is important to examine, for example, the phosphorylation levels of the Lats2 target protein such as YAP, using Phospho-YAP (Ser127) antibody, which is one of the key players of the Hippo pathway.
We thank the reviewer for this insightful comment. Our data suggested that LATS2 stimulated cell growth in NPC cells, but the precise mechanism for this stimulation was not clear. LATS2 may have regulated cell growth by dysregulation of the Hippo pathway or another pathway. This is an interesting point and deserves further study.

Since this is an easy and essential experiment, the authors should perform it. I would suggest to purchase from, for example, Cell Signaling Company the anti-phospho-YAP (Ser127) antibody and the anti-YAP antibody to perform immunohistchemistry using the patients' samples.

Criticism #4:
Fig.1C. However, no data are presented that confirm the authenticity of the immunostained signals as Lats2 itself. The authors should perform peptide competition in
immunohistochemistry and siRNA mediated LATS2 knockdown in the cell lines and confirm the disappearance of these signals in order to show that these antibody signals are not derived from background or nonspecific recognition of other proteins.
This query is posed because Lats2 localizes in the nucleus only during M phase, but these cells are mainly at the interphase. It seems to me that these immunostained images are not from Lats2 proteins.
To ensure the specificity of the primary antibodies, consecutive sections were incubated either in LATS2 primary antibody or with a non-immunized goat IgG antibody. No immunostaining was detected with the control IgG antibody (Supporting Fig.1).
This explanation does not exclude a possibility of the antibody’s recognition of unrelated proteins in the nucleus.
Transfection with LATS2 siRNA1 caused a dose dependent decrease in LATS2 protein levels 72 h post-transfection (Fig. 4C). These results show that the antibody signals are not derived from background or nonspecific recognition of other proteins.
Fig. 4C is western blot. I am talking about the immunohistchemistry. If the authors have siRNA samples, it is an easy experiment to perform it using the same set of samples.
Mitotic figures are rare in NPC, which makes it difficult to determine when cells are at M phase or at the interphase. Localization of LATS2 in the nucleus was also shown in human prostate tissue (Powzaniuk M, et al., 2004).
They also ignored the confirmation of the specificity of the antibody. I do not understand why the data was allowed to be published without such essential data.
Level of interest: An article whose findings are important to those with closely related research interests

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.