Reviewer’s report

Title: Sema3A Drastically Suppresses Tumor Growth in Oral Cancer Xenograft Model of Mice

Version: 0 Date: 12 Jan 2017

Reviewer: Constantinos Mikelis

Reviewer's report:

In the present study the authors demonstrate that Sema3A is a potent inhibitor of tumor growth and angiogenesis in oral cancer, working on the squamous cell carcinoma cell line SSC-9. Sema3A shows reduced expression levels in the SSC-9 cell line in addition to R40LN and R40P cell lines. Sema3A blocks tube formation of HUVEC and angiogenesis in the in vivo CAM assay model. The authors successfully overexpressed Sema3A in the SSC-9 cell line and performed tumor growth xenograft experiments. Sema3A overexpression blocked tumor growth and tumor-induced angiogenesis. Furthermore, the authors tried to dissect the molecular pathway in the tumor samples, showing decreased phosphorylation levels of VEGFR2, Src and FAK with Sema3A overexpression.

Overall, this is a well-structured study which achieves to clearly demonstrate the potent anti-angiogenic and anti-tumorigenic effect of Sema3A in oral cancer. Although the role of Sema3A has been already demonstrated in other cancer types, this manuscript expands this knowledge on oral cancer as well. However, there are a few remarks that could potentially improve the quality and strengthen the significance of this manuscript:

Major issues:

* Fig 3A: In the tube formation experiments and also in the methods is not described whether the HUVECs have been previously starved (only that the cells were in GF reduced matrigel). Starvation should be clearly mentioned, so that is clear whether the basal angiogenesis levels are due to autocrine effect or due to FBS growth factors.

* In Fig 3A it is clear that Sema3A blocks angiogenesis. However, should starvation occur it would be better to add VEGF as a stimulating growth factor, thus two more groups in the experiment: VEGF and VEGF + Sema3A combination. This would verify the specific role of Sema3A on VEGF signaling.

* According to current knowledge tumor angiogenesis is stimulated from cancer cell-derived growth factors acting to the adjacent endothelial cells. On this regard, an interesting complementary experiment would be to treat starved HUVEC in the matrigel assay with starvation medium from SSC-9 with and without Sema3A overexpression.
At the CAM angiogenesis assay in Fig 3B: Measurements of the control and the Sema3A group should be included. The same goes for the picture of the Sema3A alone. This would provide information whether Sema3A just blocks VEGF-induced angiogenesis in this model or angiogenesis blockade goes below the control levels.

In Fig.5 the authors discuss the "interaction between Sema3A and VEGFR2", however there are no experiments showing interaction (i.e. immunoprecipitation, colocalization) included in the manuscript. Such experiments and data should be included to discuss a possible interaction between Sema3A and VEGFR2. Therefore I believe the authors should change the description from "interaction..." to "effect of Sema3A on VEGFR2 phosphorylation levels". Alternatively, they can show interaction data.

Minor issues:

* Fig.5: The authors show phosphorylation levels of VEGFR2, Src and FAK without or with Sema3A overexpression. However, it is not clear how Sema3A overexpression leads to this phosphorylation of the VEGFR2 signaling pathway. A nice complementary approach would be to include VEGF stimulation in the SSC-9 cell line and show increase of phosphorylation levels on the VEGFR2 pathway with/without Sema3A overexpression.

* On Fig 2A should be mentioned on the figure which of the western blots shows Sema3A and which actin.

* Fig.2A has to be before Fig.1, either in the same figure or separate. Alternatively, Fig.1 could go as a supplement.

* Page 12, line 15: there is no Fig 4G. Instead the authors mean Fig. 4E I guess.

* SSC-9 is a tongue cancer cell line. Although Xenograft models are still used, it is important to mention in the manuscript the importance of the orthotopic model which has been developed and used. In this model, cells are inoculated in the tongue. We have seen different outcomes of the same cell lines in the Xenograft and orthotopic models.

* Title has to be corrected: It is a "Mouse Oral Cancer Model"

**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:
Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report
including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal