Author's response to reviews

Title: Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses vasculogenic mimicry and proliferation of highly aggressive pancreatic cancer PaTu8988 cells

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Author's response to reviews: see over
Dear Editors of *BMC Cancer*

It is indeed our great honor to submit this revised manuscript (2025797483103414R1) entitled *“Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses vasculogenic mimicry and proliferation of highly aggressive pancreatic cancer PaTu8988 cells”* to *BMC Cancer* again.

We would like to thank the reviewer and the editor for their comments and suggestions; we have made corresponding changes according to those comments. See the revised manuscript and attached figures.

The following is our responses to the reviewers’ comments.

We are looking forward to hearing the favorite decision from you.

Thank you very much.

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**Responses to the reviewers’ comments**

Reviewer’s report
The authors show that SAHA, a histone deacetylase inhibitor, inhibits pancreatic cancer cell survival and vasulogenic mimicry. In particular SAHA induces G2/M arrest, cyclin B1 degradation, p21/p27 upregulation and finally Sema-4D down-regulation associated with AKT inhibition.

In my opinion the study of the effects exerted by SAHA on pancreatic cancer cells is very interesting. However some important aspects have not been focused with accuracy by the authors. In particular it is not clear the mechanism through which SAHA caused the death of pancreatic cancer cells.

In the discussion the authors report that “one key signaling pathway that is frequently over-activated in pancreatic cancer is AKT/mTOR, which is responsible for cancer cell survival, proliferation, migration and metastasis”. In addition the authors report in the results that SAHA (10 and 20 µM) significantly inhibited activation of AKT. These considerations strongly suggest that survival and proliferation of pancreatic cancer cells is under the control of the over-activated AKT/mTOR pathway while SAHA inhibited this pathway and consequently survival and proliferation of
pancreatic cancer cells.

In order to demonstrate this thesis it is necessary to ascertain the effect of SAHA on: the level of mTOR, the level of phospho-p70S6 kinase, which is an important substrate of mTOR, the level of ULK1 which controls activation of autophagy. (Major compulsory revisions)

The results could allow to clarify the modalities through which SAHA is capable of inhibiting the survival of pancreatic cancer cells.

**Response:** the reviewer has brought a very good point which we omitted. As instructed, we have included the requested data, which showed that SAHA (10-40 µM) significantly inhibited mTOR and its downstream target p70S6 kinase (S6K1) phosphorylation/activation (see revised Figure 6A and B). However, ULK1, regular S6K1 and mTOR expression was not affected by SAHA (see revised Figure 6A). Based on these results, we suggested that Akt-mTOR inhibition might be involved in SAHA-mediated inhibition of survival/migration of pancreatic cancer cells.

In the current study, Dr. Xu and his co-authors discovered that report the vasculogenic mimicry formation in cultured human pancreatic cancer cells, showed that the HDACi SAHA executes a significant anti-VM efficiency in the progressive pancreatic cancer cells. Akt inhibition and Sema-4D down-regulation were proposed as the signal mechanisms.

Overall, this reviewer feels this is a well-organized study, and the results were interesting and convincing. The English writing of this paper is fine.

I only have a few minor points.
Please go back and check the English writing of this paper, it could be further improved, pay special attention of some of the typos.

**Response:** We have gone through the manuscript a few times and change all the typos, the English writing of the revised manuscript is much improved. Thanks.

Figure 6, please also include non-phospho-kinases for all the blots.

**Response:** Samples in Figure 6 were re-run, and non-phospho-kinases were included (See revised Figure 6). Thanks.

Thanks.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)
I have two comments for the authors as follows:
1. On page 8, the authors need to provide the rationale of “focusing on Akt signaling”.
Response: Since previous studies have confirmed that Akt and its downstream mTORC1 is important for both survival and migration of pancreatic cancer cells [1-7], we thus wanted to know whether SAHA could affect activation of Akt-mTORC1 in PaTu8988 pancreatic cancer cells. Also, it has been suggested that Akt signaling is linked with cancer cell VM [8, 9], we tested whether this signaling pathway was important for Sema-4D expression. Thanks.

2. For the scratch assay of cell migration and vasculogenic mimicry, the investigators need to show whether the cells are viable at the time of the assays. In other words, how do they know that the inhibitory effects of SAHA on cell migration and vasculogenic mimicry are not indirect and secondary to decreased viability.

Response: For the cell migration and vasculogenic mimicry assay, the PaTu8988 cells were treated with indicate concentration of SAHA for 24 hrs, at this time point, we failed to see a significant decrease of cell viability (tested by MTT assay) by SAHA (See revised Figure 4C). From the migration images, we also observed equivalent amount of live PaTu8988 cells left after indicated SAHA treatment (see Figure 4B). We are sorry for the mislabeling for the migration assay, it should be 24 hrs but not 48 hrs (see corrected Figure 4B and corresponding legends). Most of cells were dead by 10-20 µM of SAHA at 48 hours (See Figure 1). Further, to exclude the possible interference from cell proliferation, for the cell migration assay, mitomycin C (10 µg/mL, Sigma) was always added.

References:


