Author's response to reviews

Title: Ephrin-A1 Inhibits NSCLC Tumor Growth via Induction of Cdx-2 a Tumor Suppressor Gene

Authors:

Bhagyalaxmi Sukka-Ganesh (bhagyalaxmi.sukkaganesh@medicine.ufl.edu)
Kamal A Mohammed (mkamal@medicine.ufl.edu)
Frederic Kaye (fkaye@ufl.edu)
Eugene P Goldberg (egold@mse.ufl.edu)
Najmunnisa Nasreen (nnasreen@medicine.ufl.edu)

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Author's response to reviews: see over
We thank the reviewer for the valuable comments on our Manuscript MS: 8105501896555942: entitled “Ephrin-A1 inhibits NSCLC tumor growth via induction of Cdx-2 a tumor suppressor gene”. We have fully addressed the comments and the responses are incorporated the text of the revised manuscript. We believe that incorporating all the suggested changes has strengthened our manuscript.

Comment: In the previous review several criticisms were expressed many of them related to the overall scientific quality. In general the authors have invested some efforts to improve their work, including the English of the text and removal of preliminary comments.

Response: We thank the reviewer for finding the revised manuscript improved.

Comment: However, some important controls such as i) ELISA for EFNA1, ii) controls for the quality of knock-down and iii) over expression of EphA2 are still lacking.

Response: We concur with the reviewer. We regret for not making it clear. (i) We have now added the EFNA1 data. We determined the ephrinA1 expression in all the NSCLC cell lines by Western blot analysis. The new data appears in Figure 1A) in the revised manuscript. The data are discussed under results section and it appears in paragraph 2 on page 10 in the revised manuscript.

(ii). We regret for not including all the controls for the quality knock-down. We have now also added the controls to assess the knockdown of gene. The new data has been added to Figure 4 as panel B in the revised manuscript. We have included the plasmid pcMV-\(cdx2\) to over express the \(cdx-2\) gene and the expression of claudin-2 was determined by quantitative real time PCR and Western blot analysis as suggested (Figure 4, panel A and B). These data has been discussed in the results section and it appears under paragraph 1, and lines 4-8 on page 14 in the revised manuscript.
In addition the proliferation rate and tumor formation was also determined in A549 cells transfected with pcMV-cdx2 and the data appears as panel C in Figure 5; and the tumor formation data was also included in the revised Figure 6. The new data appears on page 15, and paragraph 1 and 2, in the revised manuscript.

(iii) We have now revised the Figure 2 panel-C. A549 cells were transfected with pcDNA-EphA2 to over express receptor EphA2 and the expression of claudin-2 was determined. The new data appears as Panel-C of Figure 2. The data has been discussed under results section on page 11 paragraph 2, and page 12 paragraph 1 in the revised manuscript.

Comment: The overloading of the Western Blots has not been corrected thus preventing any reasonable semi-quantitative analysis of the results.
Response: We regret the error. We have now addressed this in the revised manuscript to show the equal loading of protein and appear as Figure 3 panel-C in the revised Figure-3.

Comment: In addition, the scientific discussion of their results is narrow not taking into account the broad biological effects of Ephrin signaling and the multiple way claudins can be regulated. Therefore, I cannot express my satisfaction with the author’s improvements and would again vote for including all controls as already demanded in my first review in order to end up with a manuscript fulfilling the requirements of good scientific practice. In addition, I would suggest that the authors improve their discussion by critically reflecting their own data and considering a wider context of their findings.
Response: We have now revised the discussion in the revised manuscript to reflect the findings as suggested by the reviewer. The changes in the discussion have been marked in red in the revised manuscript.