Reviewer's report

Title: Downregulation of Cyclophilin A by siRNA diminishes non-small cell lung cancer cell growth and metastasis via the regulation of matrix metallopeptidase 9

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Reviewer: Valentina Zuco

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Comments to the Authors

The authors demonstrate that the knockdown of Cyclophilin A decreased metastatic activity in two human NSCLC cell lines. The authors report interesting novel findings on the role of cyclophilin A in the invasion and migration of A549 lung adenocarcinoma cell line and of 95 C large cell lung cell line.

Major Compulsory Revisions

1) The data reported in the manuscript does not add much to the well-known knowledge in this field. The manuscript doesn’t explain any mechanism concerning the tumor growth inhibition and the decrease of tumor invasion caused by the knockdown of CypA. Your research is pretty interesting, but unfortunately there are any explanation about the final results that you are claiming. I suggest you to go deeper in your research in order to complete your work.

2) Clarification of the quantitative Real Time PCR method. The normalization approach employed for quantitative Real Time PCR should be explained in a clearer way. In materials and methods paragraph and in figure 1 legend the authors say: “relative mRNA levels are presented as 2^[Ct(b-actin)-Ct(gene of interest)]”. What does that mean? Have they used the ##Ct approach? What is “1” in the y axes of graphs in figure 1A?

3) It’s extremely important to know protein levels of CypA in cell lines of the figure 1A. Western-blot analysis of the CypA protein levels, it is recommended.

4) Staining of cells in the invasion assay should be performed after 24h and not 48h. Thus, it is necessary to repeat this assay after 24h of incubation.

5) In invasion and migration assay, usually conditioned medium was added to the lower chamber. Are there any reason why are you using a medium containing 10% FBS?

6) To indicate the doubling-time of cells in figure 1B, 2C and D.

7) I’m not agree with your claim in page 8, line 17-18 (our data demonstrate that the suppression of CypA resulted in marked inhibition of tumor formation) and in page 9, lines 20-21 (our data indicate that CypA plays a CRUCIAL role in the progression of NSCLC). The results reported in your manuscript are not supporting your conclusion. There are not any real and objective results that can provide the involvement of CypA in the inhibition of tumor formation and the
crucial role of CypA in the progression of NSCLC. In fact, your results clearly show an inhibition of the tumor growth and not of the tumor formation.

8) The reference 22 in page 10, lines 20, is not corrected: the reference 20 is related to breast cancer. The reference 22 is related to metastatic melanoma cell lines. Please, to check references.

9) Style and language may be checked carefully.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**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