Reviewer's report

Title: Suppression of the Epithelial-Mesenchymal Transition by SHARP1 Is Linked to the NOTCH1 Signaling Pathway in Metastasis of Endometrial Cancer

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Reviewer: Marco Pupo

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In this manuscript, Liao et al. provide evidence on the involvement of SHARP1 in repressing Epithelial-mesenchymal transition (EMT) through the attenuation of NOTCH1 signalling pathway in endometrial cancer (EC). To address this aim, the Authors used two different endometrial cancer cell lines stably transfected with a lentiviral vector expressing human SHARP1 to perform in vitro and in vivo migration assays. Moreover, the authors analyzed the expression of diverse markers of EMT and NOTCH1 signalling pathway in endometrial cancer cells transfected with SHARP1 to prove the involvement of SHARP1 in repressing both EMT and NOTCH signalling. Thereafter, the Authors analyzed the expression of the EMT and NOTCH signalling activation markers in endometrial cancer cells co-transfected with SHARP1 and the intracellular NOTCH domain to confirm the involvement of NOTCH1 in the repression of EMT and cell migration elicited by SHARP1. Finally, the Authors corroborate their results performing IHC experiments on endometrial cancer and EC Lymphatic metastasis specimens and they correlate the low levels of SHARP1 in metastatic EC tissues with loss of epithelial markers and induction of mesenchymal markers.

The study deals with an interesting issue regarding the new role of SHARP1 in repressing EMT and endometrial cancer metastasis. However, the manuscript presents major and minor concerns as detailed below.

Major Compulsory Revisions

1. In figure 2 panel D Authors show the repression of NOTCH signalling pathway in Ishikawa cells transfected with SHARP1. However, regarding the expression NOTCH1 the authors do not report if they have analyzed the expression of full-length NOTCH1 or its active form NOTCH1 intra-cellular domain (N1ICD). Presumably, Authors show the expression of full-length receptor while also the expression of N1ICD should be analyzed as marker of the receptor activation. Moreover, in the same panel of the same Figure the Authors should confirm the repression of NOTCH1 pathway also in HEC-1B cells transfected with SHARP1 as showed in panel C of Figure 2 looking for the EMT markers.

2. In Figure 2C Authors should better explain how they quantify the immunofluorescence data. How many random fields were counted? How they quantified the intensity of the staining? Moreover the immunofluorescence experiments should be also performed in HEC-1B cells to confirm the data shown in the panel B of Figure 2.
3. In Figure 3 Author should confirm the activation of NOTCH1 pathway in IshikawaSHARP1 also evaluating the expression of Hes-1 which is one of the most important NOTCH1 target genes. Moreover, the Authors in the same figure should also block the NOTCH1 pathway using #-secretase inhibitor in IshikawaSHARP1 co-transfected with ICN to better confirm the involvement of this pathway in the action of SHARP1 in these cells.

4. In Figure 4 Authors should also show the results of the IHC without primary antibody as negative control for their results.

5. In the results section paragraph "SHARP1 overexpression suppresses the NOTCH1 pathway in EC cells" and again in the discussion section Authors refer to Jagged-1 as a NOTCH1 target gene. This is completely incorrect because Jagged-1 is one of the Notch ligands as the Authors themselves state in the introduction section. This need to be addressed.

Minor Essential Revisions

1. In the results section is not clear the number of specimens used for the semi-quantitative IHC particularly comparing the number of specimens with the graph in Figure 4B. Probably, inserting data of specimens used into a table could make them more comprehensible to readers.

2. In the paragraph "Verification of the SHARP1 effect on the Notch/EMT pathway in EC tissue specimens" the authors do not mention the expression of N-Cadherin which is anyway showed in Figure 4 C

3. In Figures 1A, 2B, 2C, 2D and 3A authors should show the quantification of data obtained through wound healing, western blotting and immunofluorescence experiments.

4. The authors should improve the quality of the E-cadherin immunofluorescence porthographs showed in Figure 2C. Moreover, the authors should also improve the quality of WB of SNAIL in Figure 2B, Jagged-1 and Hes-1 in Figure 2D and again Snail in Figure 3A.

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