Reviewer's report

Title: Prolyl-4-hydroxylase alpha subunit 2 promotes breast cancer progression and metastasis by regulating collagen deposition

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Reviewer: Carmen Chak-Lui Wong

Reviewer's report:

Major Compulsory Revisions

The overall quality of the manuscript is satisfactory. This manuscript has clearly conveyed the message regarding the association of P4HA2 and collagen deposition in human breast cancer tissues. This work also demonstrated the biological impact (in vitro and in vivo) upon genetic knockdown and pharmacologic inhibition of P4HA2 in breast cancer model. Similar work on the roles of P4HA2 in breast cancer has been reported by Gilkes DM et al. this year. Although this current manuscript seems to be only incremental, Xiong et al. has used a 3D culture model which is quite valuable for studies on ECM/ECM proteins.

There are a few points that the authors should address for publication:

Major:

(1) Authors fail to describe the experimental details about the setup of 3D culture, e.g. what components/gel they used, how long they embedded the cells for subsequent analysis.

(2) Figure 1. Authors categorize patients into P4HA2 low and high groups. What is the cut-off value for this categorization? Are the results consistent when different cut-off values are used?

(3) Authors should clearly explain how they grade the degree of polarization. It seems to me that this assay is only qualitative. Readers may not know how to interpret polarity of cells. Authors are suggested to provide a picture showing polarized cells and a picture of unpolarized cells. Or use arrows in the figures.

(4) Typical transwell cell migration assay with Boyden chamber should be performed to examine the effect of -control and -shP4HA2 cells in breast cell migration.

(5) Can the authors observe lung metastasis from orthotopic model? A number of breast cancer cell lines can metastasize to the lungs when implanted into the mammary fat pad, why did the authors use tail vein injection for the last experiment?

(6) Discussion/conclusion is too short. Authors should discuss about the implications of the study, how this study is going to impact/benefit the current therapeutic regimen of breast cancer, any preclinical studies on collagen
synthesizing and modifying enzymes, the importance of ECM, how the ECM generated from P4HA4 positive breast cancer cells affect the motility of the cancer cells, how the branching/ polarized properties of the cells affect cell movement, what future studies can be initiated from this work etc…..

Minor:
(7) Figure 5B and 5C are mislabeled

**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