Reviewer's report

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

**Version:** 3  **Date:** 9 October 2013

**Reviewer:** Anke Zieseniss

**Reviewer's report:**

Gaofeng Xiong et al., found P4HA2 expression levels to be associated with poor prognosis in breast cancer and conducted experiments to characterize the role of P4HA2 in breast cancer progression. The knockdown of P4HA2 in several breast cancer cell lines reduced malignant phenotypes and collagen deposition. Furthermore, the knockdown of P4HA2 in MDA-MB-231 cells suppressed in vivo tumor growth and lung metastasis.

However, this manuscript does not provide substantial new findings as many of the results only confirm findings of Gilkes et al., published earlier this year.

**Major Compulsory Revisions**

1. I am afraid that it has already been established that P4HA1 and P4HA2 are critical for collagen deposition by breast cancer cells (Gilkes et al., 2013, Cancer Res.). Also, it was shown by Gilkes et al., that the knockdown of P4HA2 in MDA-MB-231 cells inhibits tumor growth and metastasis and it was shown that P4HA2 gene expression levels are associated with decreased survival of breast cancer patients. Furthermore, Gilkes et al., show and discuss the therapeutic potential of inhibitors of collagen prolyl hydroxylases.

Thus, Gaofeng Xiong and co-authors should thoroughly work out the novelty of their findings and discuss the paper of Gilkes et al. in greater detail.

2. The authors should point out clearly that not only the activity of P4HA2 is inhibited upon 1,4-DPCA treatment and discuss their results accordingly.

3. Figure 2/3.: P4HA2 knockdown efficiency is shown in T4-2 cells. Please also provide the immunoblot data for the other cell types (ZR-75, MDA-MB 157, MDA-MB 2314).

4. Figure 5A: Please show equal loading of control and knockdown cells (e.g. Ponceau S staining). Why was this experiment done with T4-2 cells and why is collagen deposition not shown for MDA-MB cells? Particularly with regard to the experiments in Figure 6 and the general conclusion drawn by the authors “our data demonstrate that P4HA2 is required for collagen secretion and deposition by breast cancer cells” please include a justification why the experiment in Fig. 5A was performed with T4-2 cells or include data on MDA-MB cells.

5. It is unclear why the proliferation of T4-2 cells in Fig. 2 (~55 %) is different from
the proliferation of control cells shown in Fig. 4 (~10%). What is the explanation?

Minor Essential Revisions

1. Please provide more details in Materials and Methods on:
   - Sh-vector: was an empty control vector or a vector with a scrambled sequence used?
   - How was equal loading of protein for immunoblotting controlled?
2. Where are the arrows in Fig. 6 D,E,G pointing to? Provide a short description.
3. Data in Figures 5A, 6: Were the experiments performed with shP4HA2-1 or shP4HA2-2?

Minor issues not for publication

1. Figure 6B: labeling of the graph: (mm3) please use superscript 3
2. Introduction, 2nd paragraph: there is a comma missing between chondrocytes and osteoblasts
3. Materials and Methods, first and second heading: remove period at the end
4. Results and Discussion: Please control spaces before the references
5. Results and Discussion, second heading “Inhibition of P4HA2 suppresses the malignant phenotypes of breast cancer cells in 3D culture.”, first paragraph: reduced colony size is sown in Fig. 2B,C,D (not Fig. 2B, C, E) cell proliferation is shown in Fig. 2E (not in Fig. 2D).
5. Figure legends: make uniform headings (either bold characters or standard characters)

**Level of interest:** An article of limited interest

**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.