Author’s response to reviews

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

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Author’s response to reviews: see over
Dec 10th, 2013
Dr. Britta Weigelt
Associate Editor
BMC Cancer

Dear Britta,

Enclosed please find our revised manuscript (ID: 1104395371078333) entitled “Prolyl-4-hydroxylase α subunit 2 promotes breast cancer progression and metastasis by enhancing collagen deposition” to be considered for publication in BMC Cancer. We are pleased that reviewers 2 and 3 agreed that the revised manuscript were significantly improved and ready for publication. The reviewers 1 and 4 still have several minor comments. Below, we address the reviewers’ comments (in italics) point-by-point with exact changes. We hope you find the manuscript is ready for publication.

Included in the submission is: one file comprising the Manuscript and 6 Figures.

Best regards,

[Signature]

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Response to reviewer’s comments

We thank the reviewer 3 and 2 for their agreement for accepting this manuscript. We also thank the reviewer 1 and 4 for their thoughtful and helpful comments.

Following is our responses to the comments.

Referee: 1

Major Compulsory Revisions

Comment 1: The P4HA2 knock out efficiency is, in the revised manuscript, shown in T4-2 cells (Fig. 2) and in MDA-MB 231 cells (supplemental Fig. 2). However, there are no Western blots shown for ZR-75 and MDA-MB157 cells. Please provide additional data.

Response: We have included the western blotting results showing P4HA2 knockdown efficiency in ZR-75 and MDA-MB-157 cells (See supplemental Fig. 3).

Comment 2: Overall the P4HA2 protein levels seem to be very low in MDA-MB 231 shcontrol cells (supplemental Fig.2). Thus, the knock out efficiency is hard to judge and seems to be not very sufficient. P4HA2 is induced in hypoxia. Therefore, adding a Western blot showing P4HA2 protein expression in sh control cells compared to knock out cells in hypoxia might display the knock down more convincingly. Can the authors provide an explanation why the basal levels of P4HA2 seem to be so different in MDA-MB 231 cells compared to T4-2 cells?

Response: We have quantified the knockdown efficiency in shP4HA2-1 (63%) and shP4HA2-2 (43%) infected MDA-MB-231 cells (See supplemental Fig. 2). Culturing the cells in hypoxia condition may enhance P4HA2 expression, but most of 3D experiments in this manuscript were performed in the regular culture condition. It has been reported that breast cancer is very heterogeneous, thus it is not surprising to see that T4-2 and MDA-MB 231 cells have different levels of P4HA2. Microarray data show that the T4-2 and MDA-MB 231 cells have similar levels of P4HA2 mRNA (Kenny PA et al Mol Oncol 2007), thus P4HA2 may be regulated at the protein level in these two cell lines. But why the basal levels of P4HA2 are different in MDA-MB 231 and T4 cells is still unclear. We respectfully point out that those questions should be addressed in future.

Referee: 4
Minor Essential Revisions

**Comment 1:** Rephrasing my previous comment 5 for clarification: Does silencing of P4HA2 also change the grape-like structure of ZR-75-1 to a more polarised spheroid structure?

**Response:** We are sorry for the oversight. Under 3D culture condition we used, majority of ZR-75-1 cells formed round or mass structures, and silencing of P4HA2 significantly reduced the colony size, but has little effect on the cell polarity.

**Comment 2:** On page 11, please amend the 3D structure of T4-2 cells to mass-like structure as the text states grape-like structure (Kenny PA et al Mol Oncol 2007).

**Response:** Thank the reviewer for point this out, we have changed it to mass-like structures.

**Comment 3:** The abbreviations used for Figure 1D are not in line with the figure legend. Please correct BC to IBC.

**Response:** As reviewer suggested, we have changed “BC” to “IBC” in Figure 1D.

**Comment 4:** Could the cell lines MDA-MB-231 and MDA-MB-157 be written with hyphens throughout the manuscript as this is inconsistent throughout the manuscript.

**Response:** We have used “MDA-MB-231” and “MDA-MB-157” throughout the manuscript.

**Comment 5:** Please use hyphen for ER-positive, ER-negative, ERBB2-positive etc.

**Response:** We have used “ER-positive, ER-negative, ERBB2-positive, ERBB2-negative” in result (See page 10), figure legend (See page 25) and Figure 1.