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: John Muschler

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This work investigates roles of the collagen-modifying protein prolyl-4-hydroxylase alpha subunit (P4HA2) in breast cancer growth and metastasis. P4HA2 functions in proline hydroxylation in collagens, which is necessary for collagen triple helix formation and stabilization.

The authors first mine existing human gene expression datasets to demonstrate that P4HA2 expression is elevated in invasive breast cancers, higher-grade breast cancers, and ERBB2 positive breast cancers. Higher P4HA2 is also correlated with reduce patient survival. Gene knockdown by siRNA resulted in reduced tumor cell growth in 3D cultures, as did cell treatment with a broader-spectrum hydroxylase inhibitor. Likewise, collagen-1 and collagen IV synthesis were also reported to be reduced by P4HA2 siRNA and inhibitor treatment. Finally, xenograft experiments showed that P4HA2 knockdown reduced overall tumor growth and collagen deposition, and reduced lung metastasis following tail vein injections.

This manuscript addresses an important topic in cancer research. Collagen-1 over-expression and cross-linking are known promoters of cancer progression, and perturbation of P4HA2 function (and related Prolyl-4-hydroxylases) is one potential mechanism to disrupt collagen deposition. This manuscript provides experimental evidence that exposes P4HA2 as a potentially important regulator of cancer progression through collagen stabilization, and highlights P4HA2 as a potentially useful therapeutic target.

With a few exceptions listed below, the experiments are well designed and well controlled, and the data convincing. Following the specific modifications listed below, this manuscript merits acceptance.

Major Compulsory Revisions

The experimental evidence showing reduced collagen-1 and collagen IV expression (Figure 5A) is not convincing, based on a single Western, and no quantification or statistical validation. The same is true for the in vivo assessment of collagen deposition in figure 6E. These data need to be strengthened in order to argue that P4HA2 down-regulation impairs collagen deposition.

Figure 6D is hard to interpret and needs improvement. The images appear to be of too low resolution to illustrate differences in invasiveness. The figure legend
The authors do not specify which TCGA datasets they used in their gene expression analyses, and this information must be added. Without these specifications, it is impossible for anyone to duplicate their analyses. Additionally, the TCGA datasets include microarray data and RNAseq data, but it appears that only the microarray data were analyzed. It will be useful to know if the same conclusions are produced using the RNAseq data.

The conclusion statement in the abstract exaggerates the conclusions permitted by the data. The data do not “reveal a critical role of P4HA2 in breast cancer progression”. The word “reveal” might be changed to “suggest”. The last sentence of the introduction is a more accurate statement.

Minor Essential Revisions

The authors need to be more careful in their use of the word “collagen” in lieu of specifying collagen isoforms. Obviously, collagens are a large family of proteins, with diverse functions. Yet, throughout the manuscript, the authors rely mainly on the general term “collagen”, which results in many incorrect statements. Examples: the first sentence of the abstract is only demonstrated for certain collagens; the 2nd sentence of the introduction refers to collagen as a single protein; the 4th, 5th, 7th and 8th sentences of the introduction all refer to data that are specific to a subset of collagens, and not “collagen”.

Discretionary Revisions

The writing within the results section could be tightened significantly by eliminating repetitive statements. In particular, the Results section, the first sentence of each paragraph is an unnecessary repetition of statements from the introduction or prior paragraphs.

Level of interest: An article of importance in its field

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.