Author's response to reviews

Title: Overexpression of 17-beta-hydroxysteroid dehydrogenase type 10 increases pheochromocytoma cell growth and resistance to cell death

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Version: 4
Date: 12 February 2015

Author's response to reviews: see over
Dear Editor,

BMC Cancer

Manuscript ID: 8300058271369550

**Title:** Overexpression of 17β-hydroxysteroid dehydrogenase type 10 increases pheochromocytoma cell growth and resistance to cell death

Authors: Emily A Carlson, Rebecca T Marquez, Fang Du, Yongfu Wang, Liang Xu, and Shirley ShiDu Yan

Corresponding author: Shirley ShiDu Yan

Dear Editor:

This cover letter attends the web submission of the above revised manuscript, which we wish to be published as an article in *BMC Cancer*. The paper is submitted exclusively to the BMC Cancer journal. All authors are in agreement with the contents of the revised manuscript.

Thank you for your useful comments and suggestions on the structure of our manuscript. We have revised the manuscript according to the reviewers’ suggestions, and detailed corrections are listed below point by point. All the corrections are highlighted in blue text in the revised manuscript. Additionally, we decided to use HSD10, instead of ABAD, as this is the most commonly used name for the enzyme.

**Reviewer 1**

1. Is there any difference in the quality and quantity of ABAD between the normal cells and tumor cells?

   We looked at HSD10 (ABAD) expression in T47D and MCF7 human breast cancer cells compared to MCF10A human breast cells, which is a non-tumorigenic epithelial cell line. We performed immunoblotting and qRT-PCR which revealed that the non-tumorigenic breast cells had significantly lower levels of HSD10 compared to the cancerous breast cells. The breast cancer data has not been included in this manuscript as it will be used in future studies.

2. Have you ever done any experiments to identify the expression of ABAD in other tumor cells except adrenal gland tumor cells?

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Yes, we have looked at the expression of HSD10 in other tumor cells, including mouse neuroblastoma cells (N2a), human neuroblastoma cells (SK-N-SH), and two human breast cancer cell lines (T47D, MCF7). We have not included data involving these cell lines in this manuscript because it is still preliminary findings and will be used in future studies.

3. ABAD, which is present in the mitochondrial matrix and involved in multiple aspects of metabolic homeostasis as a short-chain dehydrogenase. So here is the question: Can deregulation of ABAD inhibit the tumor cells specifically?

   From what we have observed in breast cancer cells, HSD10 expression is increased in cancer cells compared to normal cells, revealing the potential for targeting cells with higher levels of HSD10 to specifically attack cancer cells. Also, cancer is a metabolism disease, and HSD10 plays a role in metabolism, which is likely a reason why HSD10 levels are elevated in cancer cells; thus deregulation of HSD10 could lead to disruption of cancer cell metabolism. This provides us with the prospect of HSD10 as a new target for cancer.

   We have included this in the discussion section of the revised manuscript.

Reviewer 2

Major compulsory revisions:

1. The authors present the TMRM data of figure 3 as evidence for the importance of ABAD on protection from apoptosis. The authors need to demonstrate the effects of ABAD on apoptosis induction alone or after oxidative stress insult (H2O2 or TBH) using different techniques, such as:

   i. TUNEL staining and quantification
   ii. the presence of cleaved caspase-3 by Western blotting
   iii. poly(ADP ribose polymerase) cleavage by Western blotting (calculate the ratio of cleaved PARP to total PARP)

   We agree with the reviewer’s comments and have now included TUNEL staining and quantification in untreated cells and after oxidative stress insult (H2O2).

2. The authors mention the potential importance of ABAD/CypD interaction for the suppression of apoptosis caused by ABAD overexpression. The authors need to
demonstrate this interaction by immunoprecipitation by pulling down with both ABAD and CypD antibodies, as well as immunofluorescence to further show a potential interaction between ABAD and CypD.

We have now provided immunoprecipitation data supporting an HSD10-CypD interaction in the pheochromocytoma cells; their co-localization within mitochondria is further confirmed with immunofluorescence staining.

Minor essential revisions

1. In the conclusions section of the abstract, the authors mention that blockade of ABAD "may halt and/or prevent cancer growth, thus providing a promising novel target for cancer patients as a screening or therapeutic option." However, this statement is incongruous with the rest of the abstract stating the effects of ABAD overexpression on cancer growth. This reviewer feels that the conclusions section of the abstract would be better if it reiterated how ABAD supports tumor growth, followed by your statement about the potential for blockade of ABAD and use of ABAD as a potential diagnostic tool or therapy target.

   We agree with this suggestion and have revised the conclusion section of the abstract accordingly.

The manuscript has been revised according to the reviewers’ suggestions.

On behalf of all the authors,

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