Author's response to reviews

Title: Activation of SNAT1/SLC38A1 in human breast cancer: correlation with p-Akt overexpression

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Version: 2 Date: 2 March 2013

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General Responses

We’d like to thank Editors and Reviewers for their thorough review, and constructive and helpful comments and suggestions. We hope that we have adequately and carefully addressed all the points raised to their satisfactions.

Responses to EDITOR-IN-CHIEF

#1: “In order to give appropriate credit to each author of a paper, the individual contributions of authors to the manuscript should be specified in this section.”

Answer: Yes, we have specified the individual contributions of authors in the Authors’ contributions section.

#2: “We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.”

Answer: Yes, we have provided a detailed Point-by-Point response herein.

#3: “Please highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript to make it easier for the Editors to give you a prompt decision on your manuscript.”

Answer: Yes, we highlighted all the major changes in yellow.

#4: “Please also ensure that your revised manuscript conforms to the journal style. It is important that your files are correctly formatted.”
**Answer:** Yes, we did so according to your instruction.

**Responses to REFEREE #1**

#1. “In Figure 3A, it is too preliminary to draw the conclusion that the conclusion that SNAT1 functions via Akt signaling pathway. The evidence that SNAT1 resulted in decreased p-Akt level couldn’t support directly the above conclusion. Therefore, the author must address this issue and provide direct evidence, such as Akt agonist rescue assay.”

**Answer:** Yes, we agree. In our revised submission, we provided direct evidence of knocking down SNAT1 leading decreased pAkt level using Akt agonist rescue assay. The new information has been provided in **Methods, Results** and **Discussion** sections and in **Fig. 3B**.

#2. “Although the author used CCK8 assay and Colony formation assay to assess the tumor growth, it is necessary to further analyze the reason of inhibition of SNAT1 down-regulation-induced cell growth, such as cell cycle or apoptosis analysis.”

**Answer:** Yes, we agree with the reviewer. In our revised submission, we revealed that SNAT1 down-regulation induced cell growth arrest and apoptosis of 4T1 cells using Flow cytometric analysis. The new data has been added in **Materials and Methods, Results** and **Discussion** sections and in **Fig. 3E** and F.

#3. “In introduction and discussion, the roles of other amino acid transporters in cancer should be mentioned and discussed.”

**Answer:** Yes. The roles of other amino acid transporters (e.g. LAT1) in cancer were discussed in **Introduction** and **Discussion** section.

#4. “In results, the title ‘Knockdown of SNAT1 by shRNA inhibits proliferation and colony formation of breast cancer cells by blocking Akt phosphorylation’ should be revised.

**Answer:** The title has changed to “Knockdown of SNAT1 by shRNA induces cell growth inhibition and apoptosis of breast cancer cells by blocking Akt phosphorylation” according to the new data (please see **Results** section)

#5. “ In the final paragraph of results, Fig. 4 should be Fig.5.”
Answer: Yes, we are sorry for the mistake and have already corrected it.

#6. “It would be nice if the whole manuscript can be polished by native English spoken professional.”

Answer: Yes, I agree with the reviewer.

Responses to REFEREE #2

#1. “This study included 70 cases of breast cancer for TMA study from 2007 to 2011 years, but the number of cases is very small. In general, TMA study included more than 200 cases for breast cancer.”

Answer: Yes, we agree. In our revised submission, we enrolled in another cohort of 140 cases with breast cancer. The new information has been provided in Methods, Results, and Discussion sections.

#2. “In method, ‘two pathologists’ should be specified.”

Answer: Yes, we agree with the reviewer. In our revised submission, we specified and thanked the two pathologists in Methods sections.

#3. “Add the number of approval for ethical review committee”

Answer: We have added the number of approval for ethical review committee in Methods section.

#4. “In method, interpretation of IHC results for SNAT1, pAKT was performed by ‘two individuals’. ‘two individuals’ should be specified.”

Answer: In our revised submission, we specified the two individuals in the Methods section.

#5. “Is there any reference for IHC interpretation system used in this study? Add the reference if exist.”

Answer: Yes, we added the reference in the Methods section.

#6. “In general, clinicopathologic parameters for breast cancer included ER, PR, HER-2 status, ki-67 LI. Add this parameters”

Answer: Yes, I agree with the reviewer. We added these parameters and analyzed their correlation with SNAT1 expression in Results section.

#7. “In this study, there is no survival analysis for SNAT1, pAKT status. Add survival
analysis for SNAT1 and pAkt by Kaplan-Meier curve and multivariate Cox analysis.”

*Answer:* Yes, I agree with the reviewer. However, since breast cancer patients have a long term of survival, only 12 patients died of cancer in our cohort which limited the survival analysis. So we only performed Kaplan-Meier curve analysis and didn’t carry out multivariate Cox analysis. Please see the *Results* section.

#8. “Formal of table1 should be changed as below.”

*Answer:* We changed the formal of table 1 as suggested (please see *Table 1*).