Author's response to reviews

Title: Glutamate acid decarboxylase 1 promotes metastasis of human oral cancer by beta-catenin translocation and MMP7 activation

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Author's response to reviews: see over
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Dr. Dafne Solera
Executive Editor, BioMed Central Cancer
Middlesex House 34-42 Cleveland Street
London, London W1T 4LB
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Dear Dr. Dafne Solera

We are now sending our revised manuscript (original MS No.: MS 6273708491068228) entitled ‘Glutamate acid decarboxylase 1 promotes metastasis of human oral cancer by β-catenin translocation and MMP7 activation’ by Kimura et al. for consideration for publication in BioMed Central Cancer.

We wish to thank you and the referees who reviewed our manuscript for the useful comments and suggestions to improve the content. We are including a letter with the responses to the reviewer’s comments and concerns and summarizing the changes in the manuscript. The revised portions are underlined in the manuscript.

We hope that our current paper based on these responses and corrections are satisfactory for consideration for publication in BioMed Central Cancer.

We look forward to a favorable response from you.
Thank you very much for your special consideration in this matter.

Sincerely yours,

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Reviewer #1

We would like to thank the reviewer #1 who has reviewed our manuscript favorably and we appreciate the comments and suggestions to improve the content. We have revised the manuscript as indicated below to address the points raised by the reviewer.

The following are our specific responses to the reviewer.

1) According to the reviewer’s suggestion, we have added the detailed method of the protein concentration from culture media in the Methods section (page 12, lines 16-17), and also replaced Figure 3c and 5c with new figures to see pro-MMP7 bands (28kDa).

2) As has been pointed out, we already compared clinical correlation between GAD1 expression and 5-year survival of OSCC patients. Unfortunately, we could not find any clinical significance. We have also corrected errors in ‘classification of stage’ in Table 1.

3) The reviewer asked ‘How do we decide an optimal concentration of GAD1 inhibitor, 3-MPA’. Erdő (ref. 28) reported that 5-10µM of 3-MPA was used for his in vitro experiment so that we adopted 5µM of 3-MPA in our study.

4, 5) We completely agree with your comments. The correlations among GAD1, β-catenin, and MMP7 must be critical information for this scientific field. Therefore, immunohistochemistry and immunocytochemistry of β-catenin and MMP7 would be of additional interest. Actually, we are now planning those experiments, including mouse xenograft of shGAD1 cells, for our next project.

6) As pointed out by the reviewer, we have asked Santa Cruz Biotechnology Inc. for shRNA sequence of GAD1. Those sequences are the trade-secrets of the company so that we could not show them in our manuscript.
Reviewer #2

We would like to thank the referee who has reviewed our manuscript and we appreciate the comments and suggestions to improve the content. We have revised the manuscript as indicated below to address the points raised by the reviewer.

The following are our specific responses to the reviewer.

[Major points]
1, 3) We totally agree with reviewer’s suggestions. The correlations among GAD1, β-catenin, and MMP7 must be important information for our scientific field. Therefore, immunohistochemistry and immunocytochemistry of β-catenin and MMP7 would be of additional interest. Actually, we are now planning those experiments, including mouse xenograft of shGAD1 cells, for our next project.

2) According to the reviewer’s suggestion, we have quantified western blotting bands in Fig. 3a and 5a. We added detailed comments about that in the Methods section (page 9, lines 7-9).

[Minor points]
1) According to the reviewer’s suggestion, we have corrected errors in stage column in Table 1.

2) According to the reviewer’s suggestion, we have replaced ‘nuclei’ with ‘nucleus’ in the figure legends of figure 5 (page 27, line 23).

3) According to the reviewer’s suggestion, we have increased font size and figures (figures 1-6).

[Discretionary comment]
We appreciate the comments and suggestions. We hope GAD1 might play an important role in controlling tumoral invasion and metastasis in OSCC.
Reviewer #3

We would like to thank the reviewer #2 who has reviewed our manuscript favorably and we appreciate the comments and suggestions to improve the content. We have revised the manuscript as indicated below to address the points raised by the reviewer. The following are our specific responses to the reviewer.

1) According to the reviewer’s suggestion, we have added the patient information, such as age, treatment, and following time, in the Methods section (page 6, lines 18-21).

2, 3) The reviewer asked the difference of cellular functions among endogenous oral keratinocytes and OSCC cells. Since we would like to figure out GAD1 functions in the OSCC cells, we had established knockdown cells, shGAD1 and shMock in HSC2 and HSC3, and compared the cellular behaviors in this study.

4) As has been pointed out, overexpression experiments might be helpful for our data obtained from knocking-down experiment. However, since overexpression of GAD1 does not reflect the situations of endogenous cells, we did not adopt that experiment in our study.

5) We completely agree with your comments. The correlations among GAD1, β-catenin, and MMP7 might be critical information for this scientific field. Therefore, we are planning immunohistochemistry of β-catenin and MMP7 in clinical samples for next project.

6) As has been pointed out, Dr. Chuang’s manuscript is critical data for our study so that we have added his report in the Background and References sections (page 5, lines 2-3; page 22, lines 22-24). In addition, according to the reviewer’s suggestion, we have replaced Figure 3c and 5c with new figures to see not only active MMP7 (19kDa) but also pro-MMP7 bands (28kDa).

7. (1)-(5) According to the reviewer’s suggestion, we have modified Table 1.