Author's response to reviews

Title: The role of IFITM3 in the growth and migration of human glioma cells

Authors:

Bing Zhao (bingzhaodr@163.com)
Hongliang Wang (Hongliang_wangdr@163.com)
Gang Zong (Gangzong83@126.com)
Ping Li (drpingpingli@126.com)

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

We were appreciative of the careful reading and reviewing on our manuscript (MS: 1523988158101528). Accordingly, this manuscript has been revised in response to every concern or comment raised by the reviewers. The revisions made were highlighted by red in the revised manuscript. In addition, we have consulted native English speakers for paper revision before the submission this time. We hope the revised manuscript will meet BMC Neurology’s standard. Below you will find our point-to-point responses to the reviewers’ comments.

Referee 1

Minor Essential Revisions: 2.

spelling mistakes: Transmembrane Protein >>>> Transmembrane Protein (page 4)

Author’s reply: We are very sorry for the mistakes in this manuscript and inconvenience they caused in your reading. We have corrected these words. Thanks so much for your useful comments.

Referee 2

Major Compulsory Revisions

The authors should be lauded for their investigation of potentially novel involvement of the IFITM3 gene in the growth and invasion of glioblastoma, particularly insidious and treatment resistant brain cancer. However, there are some major flaws in the scientific approach and data interpretation within the manuscript which need to be addressed.

1.) Immunohistochemical studies are not properly controlled. The authors do not provide positive
(i.e. tissue with previously known IFITM3 protein expression) or negative (i.e. peptide blocking) controls for the specificity of the anti-IFITM3 antibody in this particular experimental context. It is especially concerning there is such high background staining in the WHO IV (glioblastoma) tissue. Further, the authors provide no experimental data to suggest the IHC signal detected is specific to glioma cells within the tumor tissue. Glioblastoma is a very cellularly heterogeneous tumor which includes reactive astrocytes, entrapped neurons, microglia, endothelial cells, and other cell types. Thus, it is plausible the IHC signal the authors state is specific to glioma cells, may represent expression in astrocytic cell type. It would be highly recommended to perform dual antibody experiments to demonstrate co-localization of IFITM3 immunoreactivity with one or several known glioma cell expressed proteins.

**Author's reply:** We thank the reviewer for this useful suggestion. Indeed, it is really very important to perform positive and negative control during the whole experiment to examine the specificity of the anti-IFITM3 antibody. Based on IHC analysis, we have provided positive control and negative control in which the corresponding IFITM3 antibody was replaced by PBS. Such supplement could be seen as Figure S1. Data arising from our studies have shown that IFITM3 was specifically expressed in glioma tissues. We also selected the optimum image to reflect IHC staining in WHO IV sample. Furthermore, laboratory conditions considered, we couldn’t complete dual antibody experiments to demonstrate co-localization of IFITM3 immunoreactivity with one or several known glioma cell expressed proteins. We really seek for your patience and understanding.

2.) Insufficient data to support knockdown of IFITM3 via shRNA is resulting in gene specific
effects in glioma cells. First, the authors need to demonstrate that knockdown of IFITM3 via the lentiviral shRNA construct results in decreases protein expression, to supplement the finding of decreased mRNA. Further, the authors should strongly consider the use of multiple hairpin constructs (at least one additional) to demonstrate specific knockdown of the gene in question. The specificity of knockdown would be further supported by interrogating message levels of the IFITM1 and IFITM2 genes which are known homologs with significant core sequence homology. Lastly, the authors do not clearly state whether the control shRNA is a scramble of the IFITM3 shRNA which would be the most appropriate negative control in this series of experiments.

Author’s reply: We appreciate the reviewer’s comment. As shown in revised Figure 2D, western blot analysis was used to confirm the silencing of IFITM3. Compared with uninfected and Lv-shCon infected cells, the IFITM3 protein level was significantly decreased in U251 cells infected with the Lv-shIFITM3. Moreover, we designed more than one siRNA sequence targeting IFITM3 and selected a more effective one used in the original manuscript. To demonstrate specific knockdown of IFITM3 gene, these experiments are also being repeated by using another two shRNAs (5’-CCAACTATGAGATGCTCAAGGCTCGAGCCTTGAGCATCTCATAGTTGTTTTTTT-3’ and 5’-CCTCATGACCATTCTGCTCATCTGAGTAGACGAGTAGACATGGTCATGAGGTTTTTTT-3’) against IFITM3 to get comparable results. As shown in Figure S2 B, only weak band was detected in U251 cells infected with another two shRNAs targeting IFITM3, respectively, while the high expression of IFITM3 was not affected in cells infected with Lv-shCon compared with control cells. In addition, as shown in Figure S2 C, the proliferation of U251 cells was also remarkably suppressed after infection with another two Lv-shIFITM3, respectively.
3.) The paper must be restructured in terms of the results sections. The heading of the results sections should represent clearly the findings of a series of experiments designed to support an argument within the paper, and not merely a summary of assays performed.

Author’s reply: As for the referee’s concern, we have modified the results sections in the revised manuscript.

Minor Essential Revisions

1.) On page 5, line 2, the authors refer to multiple studies performed in mice, yet only one primary research publication is cited. More supporting literature citations would be necessary to substantiate the claims made.

Author’s reply: As for the referee’s concern, we have provided more supporting literature citations in the corresponding section.

2.) The pore size (i.e. micron diameter) of the transwell inserts is not specified. This parameter is critical in the study of glioblastoma cell migration, as those transwell inserts with the small pore size (i.e. 3 microns) best assess the in vivo invasive behavior of glioma cells. This is due to the small provide extracellular spaces they provide for cells to move through, which are similar to those in the brain tissue of glioblastoma patients.

Author’s reply: As for the referee’s concern, we have provided the missing parameter in the revised manuscript. Briefly, trypsinized U251 cells were transferred into the upper chambers of the transwell plates (8.0 µm pore, Corning Costar, Cambridge, MA).
3.) Figures 1, 2, 3, and 5 which include photomicrographs need scale bars.

Author’s reply: As for the referee’s concern, we have completed the missing scale bars in Figures 1, 2, 3, and 5.

We hope that with these revisions, the paper is now suitable for publication. We really appreciate your help and look forward to your reply.

Wish you all the best!

Yours sincerely,

Prof. Bing Zhao

Department of Neurosurgery, the Second Affiliated Hospital of Anhui Medical University, Hefei 230601, China.

Tel.: 86-0551-3869502

Fax: 86-0551-3869400

E-mail: bingzhaodr@163.com