Peer Review File

Article Information: http://dx.doi.org/10.21037/atm-20-3219

Reviewer A

91- current standard therapy regimen should be included maximum safe surgical resection.

Reply: Thank the reviewer's valuable suggestion. And we have added "maximum safe surgical resection" in the manuscript.

There are many grammatical points to be corrected as below.

47 while

61 CGGA, Gravendeel

152 Japan). Kiaa0101 space

160 negative (0 point) should be points

206 A 600 μL of...

227 5% non-fat 228 milk

258 time of patients. SPSS 21.0((SPSS, Inc., Chicago, IL, USA)

272 we perform qPCR qPCR

Reply: I'm very sorry to give you so much trouble for those spelling errors. I have carefully corrected them.

Reviewer B

Reviewer´s comments

In this interesting article, the authors show the relevance of Kiaa0101 in the pathogenesis of glioblastomas. Kiaa0101 is a protein whose function has been poorly studied. Here, the authors compare its expression in different glioma grades and normal tissue by bioinformatic analysis of data in four different databases, and they compare such results in patient biopsies and cell lines. Additionally, they perform functional analysis in cell lines, and one animal model to determine Kiaa0101 involvement in glioma promotion. Although the article is of relevance and most of the bioinformatic analysis and the experimental design are well designed and performed, some issues must be clarified. English must be improved.

MAJOR COMMENTS

Introduction

- Although in the introduction section, authors describe the expression of Kiaa0101 in gliomas, there is not a clear description of its function, nor a direct relationship between such protein and Snail1, Vimentin, MMP2, and MAPK signaling pathway. Authors should address if there are studies that suggest a relation between them or to describe the rationale to study the relation between Kiaa0101 and MAPK.

Reply: Thank you very much. I have paid attention to this issue and added description of function of Kiaa0101 and its association with EMT markers. Description of relationship between Kiaa0101 and MAPK was also added.

Methods

- The authors did not indicate the antibodies dilution employed. Also, the concentration of DAPI used was not reported.

Reply: The antibodies dilution was presented in Table1 and concentration of DAPI was added in manuscript.

- There should be added the conditions of normal brain tissue collection. This is to establish if such tissue is a good control of Kiaa0101 expression in the brain.

Reply: All the frozen brain tissues were immediately stored at − 80 °C until biochemical assays

could be conducted. We mentioned these in the section of Methods (Patients samples).
- The description of the immunofluorescence is not clear. It should be re-written.
Reply: We have rewritten the description of the immunofluorescence.
- The authors did not disclose the criteria to segregate results obtained in databases between low and high expression of Kiaa0101 in order to perform Kaplan-Meier graphs.
Reply: Patients were divided into high and low groups according to the 50% cutoff point of Kiaa0101 expression in public datasets. We added the criteria of segregate in section of Statistical analysis.
Results and Discussion
- In Figure 1F it is necessary a label that identifies NBT and glioma immunohistochemistry staining: What kind of glioma or astrocytomas grade is the sample shown as a representative image.
Reply: Sorry for the negligence. We added details of samples shown as representative image.
- Gliomas are not divided into 4 grades. The classification refers to astrocytomas.
Reply: Glioma is graded I-IV according to the 2016 World Health Organization classification of central nervous system tumors. In the public datasets, gliomas of different WHO levels are included, not just astrocytomas.
- In Figure 2A-C it would be interesting if authors add a column of the expression of Kiaa0101 in the NBT samples along the databases analyzed as in Figure 1A-C. Also, authors should mention in the text or the figure legend why the expression of Kiaa0101 in grade II gliomas was only reported for one of the four databases analyzed.
Reply: Thank you for your suggestions. Figure 1 illustrates the difference between Kiaa0101 expression in NBT and glioma, and Figure 2 illustrates the correlation between Kiaa0101 and tumor malignancy (WHO grade). As a result, we only presented expression of Kiaa0101 in different grades of glioma in Figure2A-C. All public datasets were downloaded from Gliovis. WHO grade I gliomas were only reported in Gravendeel. We mentioned in the figure legend.
- In the Results and the Discussion sections, authors present a correlation between Kiaa0101 and IDH1 mutations, however, they should add the clinical relevance (or meaning) of IDH1 mutation presence in gliomas.
Reply: Thank you for your suggestions. We have added the clinical relevance of IDH1 mutation presence in gliomas in Results section.
- In Figure 2K-N it is not clear if the presented data include all grades of glioma or just high-grade gliomas (HGG). It would be interesting if the authors show if the presented results in Fig.2K-N are only valid for HGG.
Reply: Figure 2K-N showed the results of all gliomas.
- The changes in the actin cytoskeleton that authors mentioned in the Results section are not evident in the representative images they presented in Figure 3B. Maybe if they show other images or a graph about fluorescence intensity, this could be clearer.
Reply: Sorry for the inappropriate representative images due to our negligence. We replaced the representative images of actin cytoskeleton (Figure 3B).
- Changes in p-p38 are associated with changes in the activity of such protein, so the affirmation about the effect of Kiaa0101 in the expression of p-p38 must be changed in order to truly describe the effects of Kiaa0101 in terms of p38 activity.
Reply: Lentivirus-delivered shRNA gene knockdown was performed and p-p38 was obviously changed after knocking down Kiaa0101 in glioma cells. We confirmed that Kiaa0101 can affect the level of p38 phosphorylation by performing WB and IF.
- Interestingly, p38 remains phosphorylated at a very long period after Kiaa0101 overexpression, so it would be interesting if authors discuss the implications of such sustained activation in glioma cells.
Reply: In Discussion section, we mentioned that Kiaa0101 regulated the phosphorylation of p38.

Previous study had found that Kiaa0101 was mainly located in nucleus and the sustained activation might be the result of direct interaction between Kiaa0101 and p38. The nuclear export of p38 is reported to require its dephosphorylation. Because it lacks a nuclear localization sequence (NLS) and a nuclear export sequence (NES), so we assumed that Kiaa0101 might act as a Chaperone protein that involved in nuclear translocation of p38. Whether Kiaa0101 affected or how Kiaa0101 affect the translocation of p38 remained to be explored.

- It would be interesting if authors discuss their data in terms of the known information about Kiaa0101 protein structure (their domains or known functions).

Reply: Thank you for your suggestions. In the discussion section, we discussed the relationship between the Kiaa0101 function and P38. Based on your suggestions, we have added some relevant discussions.

MINOR COMMENTS

- There are many punctuations, spaces, and capital letter errors, especially when something is between parenthesis.

Reply: I have to apologize for giving you so much trouble because of those misspelling or punctuations, spaces. Your comments and suggestions really helped me a lot. I have checked manuscript carefully and I wish it can be satisfactory.

- In line 300, Kiaa0101 lacks one number 1.

Reply: We have revised.

- In Figure 3A it is not indicated which blot corresponds to which cell line, nor is indicated the percentage of silencing authors achieve.

Reply: Thank you very much. Blots corresponded to different cell lines are marked and the percentage of silencing was presented.

- In Figures 3B, 5G and 6B there is the legend DPAI instead of DAPI

Reply: We were really sorry for your careless mistakes. Thank you for your reminding.

- In Figure 5E, a label indicating which treatment is in each blot column is needed.

Reply: Thanks. We have corrected these mistakes based on your suggestions.