

Peer Review File

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Reviewer A

The paper titled “TAK-242 protects against oxygen-glucose deprivation and reoxygenation-induced injury in brain microvascular endothelial cells and alters the expression pattern of lncRNAs” is interesting. TAK-242 alters the expression pattern of lncRNAs in OGD/R cells, and differently expressed lncRNAs may exert a protective effect against OGD/R injury through a mechanism of competing endogenous RNA (ceRNA) and encoding short peptides. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply: We added related description in the revised manuscript.

Changes in the text: page 2, line 45-54.

2) What is the potential application value of TAK-242 in clinical practice? What is the basis for selecting the concentration of TAK-242 in this study? Is the dosage safe in clinical practice? Please provide literature support.

Reply: We added the potential application value of TAK-242 in the Introduction section (page 6, 161-167). Additionally, we selected the concentration of TAK-242 followed previous study (PMID: 29909454), changes see in page 7, line 202-203. Whether the dosage is safe or not need more clinical investigation.

Changes in the text: page 6, 161-167, page 7, line 202-203.

3) Figure 1 lacks statistical results for western blot, it is recommended to supplement.

Reply: Thanks for your comments. We added statistical results in Figure 1E.

Changes in the text: Figure 1E.

4) It is recommended to conduct functional studies on key lncRNAs and ceRNAs, which may make the entire study more complete.

Reply: Many thanks for your comments. This is our limitation, we added this statement in the Discussion section.

Changes in the text: page 15, line 430-433.

5) How does lncRNA interact with other signaling networks in disease progression? What dual role does it play in increasing/inhibiting tumor progression? Suggest adding relevant content.

Reply: Firstly, lncRNAs participate in multiple biological processes and exhibit various regulatory functions through epigenetic modification, transcriptional, chromatin remodeling, and mRNA integrity, and post-translational modification to regulate the mRNA expression or protein expression, which expression changes affected the downstream signaling networks. We added these in the introduction section. See page 5, line 126-130.

Secondly, lncRNAs play dual role in cancer, for example, lncRNA-PART1 inhibits the progression of glioma, but promotes the lung cancer development. We also added these contents in the Introduction section. See page 5, line 130-132.

Changes in the text: page 5, line 126-132.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “TAK-242 ameliorates epileptic symptoms in mice by inhibiting the TLR4/NF- κ B signaling pathway, PMID: 35965792”. It is recommended to quote the article.

Reply: We have quoted this article in the Introduction section.

Changes in the text: page 4, line 118-119.

7) In addition to TLR4/NF- κ B signaling pathway, which signaling pathways may this effect be closely related to? It is recommended to add relevant content.

Reply: TLR4 affected many other pathways, such as MyD88/mTOR pathway, TRIF/TBK1/IRF3 pathway, and MAPK pathway etc. Therefore, TLR4 play an important role in different diseases. We added these contents in the Introduction section.

Changes in the text: page 4-5, line 122-125.

Reviewer B

First, the abstract needs some revisions. The background did not indicate the potential clinical significance of this research focus and why the current research focus is important. The methods need to briefly describe the purposes of these experimental procedures such as the steps in the hypothesized path ways. The results need to quantify the findings by reporting statistics such as the secretion levels and accurate P values. The conclusion could be more detailed for the clinical implications of the findings.

Reply: We have revised the Abstract. However, the words in the abstract was limited between 200 and 350, we did not added secretion levels and accurate P values.

Changes in the text: page 2-3, line 45-79.

Second, in the introduction of the main text, please have a review on the known mechanisms underlying neuronal injury after surgery involving DHCA and what has been known on the roles of BMEC. The authors need to indicate the potential clinical implications of the findings.

Reply: (1) The known mechanisms underlying neuronal injury after surgery involving DHCA: Ischemia and hypoxia results in the excessive and hyperactivity of neuronal cells, which increase the expression of genes that promote oxidation and the caspase family genes to induce the apoptosis, and the adenosine triphosphate deletion leads neuronal cells necrosis. See page 4, line 93-96.

(2) The roles of BMECs: See page 4, line 98-109.

(3) The potential clinical implications of the findings: see page 6, line 174-175.

Changes in the text: page 4, line 93-96; page 4, line 98-109; page 6, line 174-175.

Third, in the methodology of the main text, please use a flowchart to briefly summarize the

experimental procedures of this study and in statistics, please ensure $P < 0.05$ is two-sided. The discussion needs to have comments on the limitations of findings from animal studies, which are difficult to generalize to human beings.

Reply: (1) Many thanks for your comments. In the methods section, each experimental method is relatively independent, just like in the previous articles (PMID: 37065546, 37065594, and 37065597), so we did not add a flow chart

(2) In our study, we used One-way analysis of variance (ANOVA) to determine the statistically significant differences among multiple groups, not two-tailed test.

(3) We added limitation in Discussion section. See page 15, line 431-434.

Changes in the text: page 15, line 431-434.

Reviewer C

1. Table S3-S5:

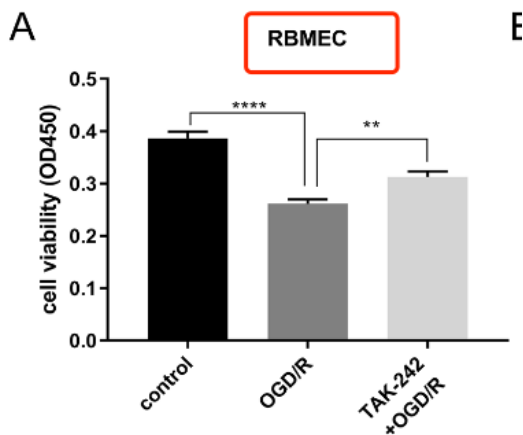
Please provide Table titles for Table S3-S5.

Reply: We added the Table title in Table S3-S5, respectively.

Changes in the Table S3-S5.

2. Figure 1:

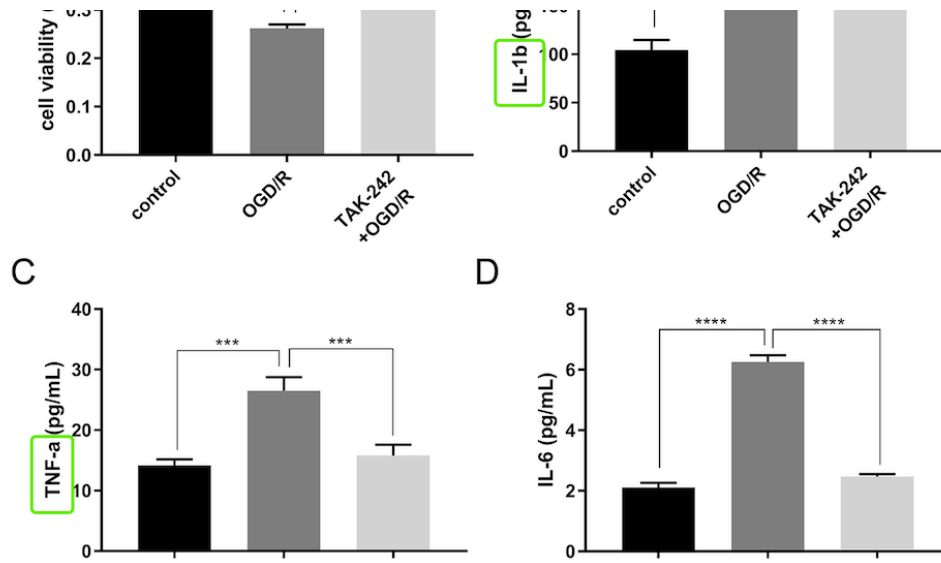
1) Please check whether the word “RBMEC” is correct.



Reply: Actually, only RBMEC cells were used in our study, and our manuscript wrote the name as BMEC cells. Therefore, we deleted the RBMEC in the Figure 1A.

Changes in the text: Figure 1A.

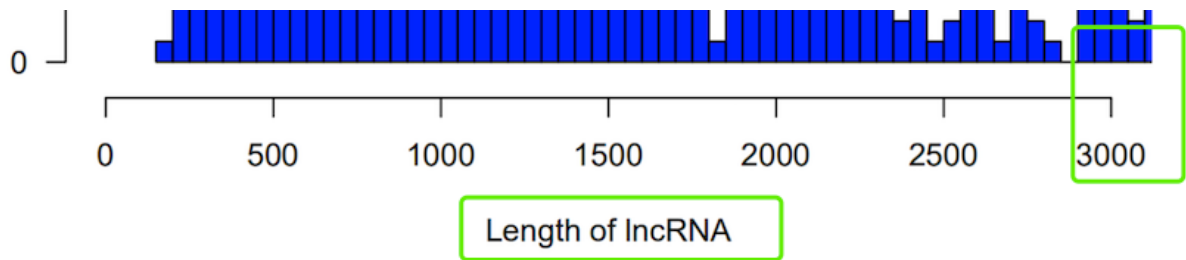
2) The below two words should be “ $IL-1\beta$ ” and “ $TNF-\alpha$ ”. Please revise.



Reply: We revised the words in the Figure 1B and 1C.
 Changes in the text: Figure 1B and 1C.

3. Figure 2:

Please add unit for the x-axis of Figure 2C and there are bars exceed 3000 in the x-axis; please modify the x-axis.



Reply: We revised the Figure 2C.
 Changes in the text: Figure 2C.