Author’s response to reviews

Title: Survival analysis of immune-related lncRNA in low-grade glioma

Authors:
Xiaozhi Li (lixz@vip.163.com)
Yutong Meng (mengyt@vip.163.com)

Version: 1 Date: 30 Jul 2019

Author’s response to reviews:

Dear Editors and Reviewers,

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Survival analysis of immune-related lncRNA in low-grade glioma” (ID: BCAN-D-19-01925). Those comments are all valuable and very helpful for improving our paper. We have studied comments carefully and have made correction and improvement which we hope meet with approval. The main corrections in the paper and the responds to the reviewer’s comments are as following:

Responds to the reviewer’s comments:

Comments in the Referee 1 in the attachment.

1. Response to comment: (1) Materials and methods. The authors should better specify and provide direct links to the dataset they used. Specifically, they should mention that it was a RNA-Seq dataset and weather it was already normalized. The authors should also specify weather the limma analysis has been applied to the whole dataset or just the genes annotated as lncRNAs.

Response: Thank you for your comment. The link descriptions of TCGA and Innate datasets are similar to the previous published articles[1-3]. Based on the reviewer's suggestion, we added the RNA-Seq database description to the newly submitted manuscript. This study used the “edgeR” package of R software to standardize the whole dataset. The similar method has been reported[4]. (Materials and Method section, line 52-55, page 3)
2. Response to comment: (2) I don't understand how the authors characterized lncRNAs as being immune-related. Specifically, what does the sentence "correlation between molecules was calculated" means in that context.

Response: Thank you for your comment. We calculated the Pearson correlation coefficient between all genes, and genes with high correlation with immunomodulatory molecules were defined as immune-related. The similar method for finding molecules with particular features by correlation analysis has been reported[5].

3. Response to comment: (3) In the last paragraph of materials and methods, variants of parametric and non-parametric two-sample tests are mentioned but never used in the paper. Moreover, I don't think that "the abnormal distribution" is the correct wording to indicate a non normal distribution.

Response: Thank you for your comment. We are very sorry for our negligence. We removed the description of the parameter and nonparametric tests, and the description whether they are normally distributed in the newly submitted manuscript. (Materials and Method section, line 76-78, page 4)

4. Response to comment: (4) Results - The authors report that at the first stage or their screening the looked for differentially expressed lncRNAs but they do not specify how they contrasted the samples (e.g. tumor vs normal, dead vs alive).

Response: Thank you for your comment. In our newly submitted manuscript, we added that we identified differentially expressed lncRNAs by comparing tumor tissue with normal tissue. (Results section, line 85-86, page 4)

5. Response to comment: (5) according to the characterization based on innatedb, there is a significant enrichment of immuno-related lncRNA in the set of overexpressed lncRNAs, that is never mentioned or discussed.

Response: Thank you for your comment. According to the threshold of correlation coefficient and P-value criteria, we found it interesting to identify more down-regulated lncRNAs than up-regulated lncRNAs in the study, which had been added in the new manuscript. (Results section, line 93-95, page 5)
6. Response to comment: (6) In the univariate Cox regression series, multiple testing was not addressed.

Response: Thank you for your comment. In this study, Efron approximation was used, and we added this description to the “Materials and methods” part in the new manuscript. (Materials and Method section, line 65-66, page 3)

7. Response to comment: (7) No technique to address overfitting has been used, such as, for example, the division of the dataset into a discovery and a validation set. Therefore, there is no evidence that these results can be generalized.

Response: Thank you for your comment. In the new manuscript, we used “caret” package of R software to randomly divide the dataset into a training cohort and validation cohort by the ratio of 7:3. The risk score was constructed using the training cohort and verified by the validation cohort. The results suggested that the risk score had good sensitivity and specificity in both the training cohort and validation cohort. (Materials and Method section, line 54-55, page 3; Results section, line 98-120, page 5-6)

8. Response to comment: (8) The authors report that, based on multivariate survival model they divided samples into hi-risk and lo-risk, but there is no mention of the threshold used (e.g. median, zero etc.).

Response: Thank you for your comment. We divided glioma samples into high-risk and low-risk groups based on the median risk score. This statement was added in the newly submitted manuscript. (Results section, line 111, page 6)

9. Response to comment: (9) The authors specify that besides the 10 lncRNAs in the survival model, there were other 7 lncRNAs that were “independent prognostic risk factors”. However, I cannot find any description of these 7 lncRNAs and the respective multivariate models, which supposedly, would have included clinical covariates.

Response: Thank you for your comment. Because of the changes of the analysis dataset grouping, the newly constructed model was based on 8 lncRNAs. We are very sorry that the previous statement was not very clear about the independent prognostic risk factors. In the newly constructed model, 7 of the 8 lncRNAs were independent prognostic risk factors for glioma, and the corresponding P values are shown in Table 1. (Results section, line 107, page 5)
10. Response to comment: (10) The authors subsequently identify genes that are differentially expressed between the hi-risk and lo-risk groups. However, these genes are never reported. Instead, they present a gene enrichment analysis that identifies mostly broad functional categories (e.g. extracellular region) and is poorly informative.

Response: Thank you for your comment. The original aim of this study was to explore the potential functions of immune-related lncRNAs in gliomas by grouping glioma samples into high-risk group and low-risk group. As a matter of fact, a total of 442 genes were differentially expressed between the high-risk group and the low-risk group, and these genes were subjected to gene set enrichment analysis. The similar method for exploring lncRNA functions has been used in previous articles[3, 6]. These enriched items may help scientists and doctors determine the directions of further research of the mechanisms by which immune-related lncRNAs affecting glioma. (Results section, line 122-131, page 6)

11. Response to comment: (11) Figures 1a,2a,3a - The color legend doesn't read well (at all). Usually, before clustering, gene expression values are centered by mean, so that the (unspecified in the paper) distance between samples, does not incorporate the scale differences between gene expression baselines but just their differential expression. This also can help to generate a visually interpretable heatmap. In addition, the samples dendrogram, does not show any feature associated with samples (like for instance a bar with color codes) and axis labels (gene names and sample names) are not readable.

Response: Thank you for your comment. In the newly submitted manuscript, we centered the gene expression by mean values when drawing the heatmap. In addition, we removed gene names and case names that could not be clearly displayed, and only clustered gene sets. (Figure1-3)

Special thanks to you for your excellent comments.
Comments in the Referee 2 in the attachment.

1. Response to comment: (1) The authors should try to briefly hypothesize the function of the identified immune-related lncRNA (in a table?)

Response: Thank you for your comment. In the final part of the results, we used the risk score based on immune-associated lncRNAs and grouped the dataset into high-risk and low-risk groups. The differentially expressed genes were analyzed by GO and KEGG enrichments. Further, the biological functions in which these immune-associated lncRNAs may be involved were identified. Similar method of this study for exploring potential functions of lncRNAs has been used in previous articles[3, 6]. This study presented the possible functions of the identified immune-related lncRNAs in the plot of gene set enrichment analysis in Figure 4. (Results section, line 122-131, page 6)

Special thanks to you for your excellent comments.

We made every effort to improve the manuscript. We appreciate for Editors/Reviewers’ hard work earnestly and hope that the correction will meet with approval.

We appreciate for your comments and suggestions sincerely.
References


