Peer Review File

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Reviewer A

N6-methyladenosine (m6A) is closely related to glioblastoma (GBM) progression. The significance of m6A modifications depends on the m6A readers, whose functions in glioma progression are largely unknown. In the manuscript "Effect of the N6-methyladenosine reader IGF2BP3 on the malignant progression in glioma", authors investigated the expression of the m6A related gene in glioma and its effect on the malignant progression of glioma.
Couple questions are required to be answered before it will be accepted.
(1) The IGF2BP3 was the crucial topic in the paper. How about the roles of IGF2BP3 in cancer? Please make a brief introduction about IGF2BP3 in the introduction.
Reply: We added a brief introduction about IGF2BP3 in the introduction.

(2) It was better to add related reference (DOI: 10.21037/gs-21-859) about the N6-methyladenosine reader in cancer.
Reply: We added the related reference in the revised manuscript.

(3) Why to choose female mice? And state clearly the body weight of used mice in methods.
Reply: We choose female Balb/c-nu mice rather than male Balb/c-nu mice because the female Balb/c-nu mice's immune system is weaker than the male's. The female mice are more easily obtained xenograft tumors. Meanwhile, male Balb/c-nu mice exhibit behaviors such as fighting and hurting others, which will cause the failure of the transplanted tumor model and the death of the Balb/c-nu mouse. The body weight of mice was stated in the methods in the revised manuscript.

(4) It was better to test the expressions of IGF2BP3 in human gliomas tissues by RT-qPCR and Western blot.
Reply: Thank you very much for your positive and constructive comments and suggestions. The human glioma tissues we collected were fixed in formalin after they were taken out, unsuitable to test the expressions of IGF2BP3 by RT-qPCR and Western blot. Our team is recently collecting clinical specimens for further validation in subsequent experiments.

(5) In the figure 3A, the length of band of IGF2BP3 was shorter than that of actin in Western blot. Why? It was better to replace it with a new.
Reply: We are very sorry for our negligence. We replaced a new one in the revised Fig 3.

(6) In the figure 4B and C, the background color of U87 and U251 group was different. Please unify the background color.
Reply: We unify the background color in the revised Fig 4.

(7) There were 10 hub genes in IGF2BP3-related DEGs in gliomas. So, it was advised to validate representative hub gene by experiments.

Reply: Thank you very much for your positive and constructive comments and suggestions. In this paper, we mainly studied the effects of IGF2BP3 on the biological functions of glioma cells. We will verify hub gene in subsequent experiments.

(8) There were many reports about the correlations between m6A and immune infiltration in cancer? What were the correlations between IGF2BP3 and immune infiltration in glioma? Please state in the discussion.

Reply: We added it in the discussion.

Reviewer B

First, the title needs to indicate the prognostic role and underlying mechanisms of N6-methyladenosine reader IGF2BP3.

Reply: Thank you very much for your suggestions. The title was modified as "N6-methyladenosine reader IGF2BP3 as a prognostic Biomarker contributes to malignant progression of glioma."

Second, the abstract needs some revisions. The background needs to indicate the clinical significance of this research focus. The methods need to describe the pathological samples in the TCGA database, the clinicopathological factors and prognosis outcomes of the 40 patients, and how the prognostic role was analyzed. The results need to quantify the findings by reporting statistics and P values including the survival rates of the two groups and correlation coefficients. The conclusion on the "therapeutic target" seems to be arbitrary since there is no intervention focusing on IGF2BP3 in this study.

Reply: Thank you very much for your positive and constructive comments and suggestions.

1.We added the clinical significance of this research focuses in the background.

2. We download and collate the RNA-seq data of the STAR process of TCGA-GBM and TCGA-LGG projects and extract the TPM format data and clinical data at https://portal.gdc.cancer.gov/.The prognostic information and clinical data of the patients are real and verifiable. Raw data can be downloaded publicly. The prognostic role was analyzed based on TCGA data which is external validation.

3. The 40 samples are from our hospitalized patients. We analyzed the expression of IGF2BP3 by immunohistochemistry in the present study. This will be an open observational cohort study, and more samples will be collected. The prognosis and survival rate are still in the follow-up and we look forward to our results as internal verification.

4. IGF2BP3 plays an important role in the malignant progression of glioma, tumor phenotype changed after we regulate its gene expression, so we speculated it might be a potential therapeutic target. Of course, more research and evidence are needed. We appreciate your detailed and professional suggestions, which require further experimental and clinical validation.

Third, the introduction of the main text needs to review what has been known on the prognostic biomarkers of GBM and have comments on their limitations to indicate the clinical needs for identifying new prognostic biomarkers. It is also necessary to describe the possible clinical implications of this research focus here.

Reply: We added these in the introduction according to suggestions.

Fourth, in the methodology of the main text, please first have an overview of the research procedures, the research methodology, the clinical factors and prognosis outcomes in the dataset of the 40 patients. In statistics, please describe the analysis of the prognostic role of IGF2BP3, the test of normality of variables, and ensure P<0.05 is two-sided.

Reply: The 40 patients just provided the samples to use as an immunohistochemical study. Clinical outcomes and clinical information are from TCGA. We briefly described the experimental method and modified it accordingly in the Methods section.

In detail, we downloaded the data and rearranged the group by IGF2BP3 expressed high and low groups respectively. We use Software: Version R (4.2.1), and R Package survival[3.3.1], survminer, ggplot2[3.3.6] Survival package was used to test the proportional risk hypothesis and fitted survival regression. The results were visualized using the survminer package and the ggplot2 package. If the best grouping method (best) is selected, the surv cutpoint function in the survminer package is used for optimal grouping cut-off screening.

Data filtering strategy: remove no clinical information or eliminate data that do not meet the normality test.

group	numbers	Total number of events	Total deletion events	Total deletion ratio	Median survival time	confidence interval
Low	348	51	297	85.3%	4068	2875-4445
High	350	2212	129	36.9%	598	535-737

The premise of the application of Cox regression is that the independent variable is required to meet the equal proportional risk hypothesis (P > 0.05), that is, the risk of the independent variable will not change with the change of time. If it does not meet the requirement, Cox regression is not suitable for testing.

Statistics (chi-square value)	degree of freedom	P
33.579	1	6.84e-09

The two group Cumulative survival rates were attached. (EXCEL format.)

Reviewer C

1. Reference should be cited consecutively, i.e. Ref. 2 should be cited between Ref. 1 and 3. Please revise.

Reply: We have adjusted the reference order.

2. Citation of Ref. 5, 23 and 35 are missing in the main text. Please revise.

Reply: We are apologized for missing that. We have revised it.

3. Please check to see if more references should be cited in the following sentence, as you mentioned 'studies'.
"Recently studies have found that the expression of m6 A regulators are related to the infiltration of tumor immune cells (40)."
Reply: We revised it to "a study".

4. Figures
-Please send us Figure 2A-C in higher resolution, as the current ones are not clear enough for publication.
Reply: It was replaced by a clear figure (Figure 2-revised)

-There is no * in Figure 5. Please check.
Reply: We checked and deleted it.

-Please explain the dots of different colors in Figure 6A, and send it to us with higher resolution.
Reply: Red represents significantly different up-regulated genes, blue represents significantly different down-regulated genes, and black represents genes with no significant difference.
And the figure was replaced by Figure 6-revised of a higher resolution.

-Lettered subparts of whole figures may be cited in any order in the text if the first mention of each whole figure is in numerical order. For example, Figure 6 contains 3 subparts (i.e.: Figure 6A, 6B, 6C). These subparts should be cited consecutively, unless Figure 6 as a whole is already cited before Figure 6A, 6B, 6C.
Reply: Figure 6 was readjusted. We rewrote this paragraph and also the figure legend.