Peer Review File

Article information: http://dx.doi.org/10.21037/tcr-20-2476.

Reviewer Comments

Major points:

Comment 1: The article should be proofread to avoid English grammar mistakes. For example, “bioinformatics analysis” could be replaced by “bioinformatics analysis” (in the title), “associated with” could be replaced by “associated to” (in the title), “removal” by “resection” (line 71), “predicting prognoses” could be replaced by “prognosis” (line 95), etc. The reading of the entire manuscript by native English or an editing system is required.

Reply 1: Thank you so much for your careful check and the labeled mistakes has been corrected in the revised manuscript. We are sorry for the carelessness. However, after consulting with native English and the editing system, it is suggested that “associated with” is much more commonly used than “associated to”. Thus, we kept the former one. The entire manuscript has been polished and proofread by an editing company and the certificate of English editing is provided in the supplementary file.

Changes in the text1: We corrected the grammar mistakes as advised (see Page1, Line2; Page6, Line77; Page7, Line103). All the corrections have been marked in red.

Comment 2: The main question on this study is the lack of characteristics of the patient cohort from TCGA database. More specifically, were primary and secondary GBM confused in the analysis? Indeed, primary and secondary GBM present different genetic alterations (in particular IDH status), as shown in particular by the revised WHO classification (Louis DN et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131(6):803-820). Thus, de novo or secondary GBM are not treated in the same way by radiotherapy and their radiological sensitivities are different. If the primary and secondary GBM were not separated in this study, so this one may be biased and lead to inconclusive results.

Reply 2: Thank you for your rigorous consideration. The patients from TCGA database were all diagnosed with primary GBM and patients with secondary GBM were excluded. To be clearer and in accordance with the reviewer concerns, we have modified the texts as "primary GBM" in the Material and Methods section.

Changes in the text2: We modified our texts be to more specific (see Page4, Line53; Page8, Line124; Page8, Line127; Page9, Line136).

Comment3: The authors focused on the radioresistance of GBM but can they confirm that the patients included in their analysis received only

radiotherapy. Perhaps GBM patients also received chemotherapy, as in the standard treatment (Stupp protocol with concomitant TMZ then adjuvant TMZ)?

Reply3: Thank you for the consideration. In this study, we focused on the GBM radioresistance. Therefore, all the GBM patients extracted from TCGA and GCGA database were treated with radiotherapy. Besides, most of the patients received chemotherapy but the detailed chemotherapeutic regimens were not provided in these databases. A small portion of the patients received only radiotherapy. Statistically, we did preform univariate and multivariate analysis to test whether other clinical parameters, such as age, gender, chemotherapy, contribute to the clinical outcome of GBM patients underwent Radiotherapy. The result showed chemotherapy was uncorrelated to the clinical outcomes of patients with GBM when they all received radiotherapy (see Table1 and 2 below).

Table 1. Univariate Analysis for Overall Survival of patients with GBM-RT

Variable	Hazard Ratio	95% Confidence Interval	*P* Value
Age	1.027	1.018-1.036	<0.001
Gender	0.843	0.665-0.069	0.160
chemotherapy	0.796	0.546-1.161	0.236
Stromal score	1.000	1.000-1.000	0.025
Immune score	1.000	1.000-1.000	0.021

Table 2. Multivariate Analysis for Overall Survival of patients with GBM-RT

Variable	Hazard Ratio	95% Confidence Interval	*P* Value

Age	1.022	1.012-1.033	<0.001
Gender	0.916	0.710-1.182	0.500
chemotherapy	0.776	0.526-1.146	0.202
Stromal score	1.000	1.000-1.000	0.483
Immune score	1.000	1.000-1.000	0.048

Comment4: It is necessary to describe and take into account the main characteristics of the patient cohorts because different covariates exist such as sex, age or the different types of treatment. It would be relevant to take these different factors to correctly stratify the patients included in this retrospective study and perhaps even include them in the statistical analyzes to be sure that these results are independent of these covariates.

Reply4: Thank you for this suggestion. We have analyzed different clinical factors of the patient cohorts including sex, age and chemotherapy, and found age and immune score were independent prognostic factors (Table 2). In this study, we focused on the effect of tumor microenvironment on GBM radioresistance. Thus, based on the immune and stromal score, the GBM patients received Radiotherapy were categorized into high and low groups; and their correlations with clinical outcomes of GBM-RT patients were further investigated.

Comment5: This study focused on the immune and stromal cells of the GBM microenvironment, but there are other components such as

endothelial cells that have an undeniable role in the response to RT. Taking the tumor vascularization into account in this study, in parallel with the ESTIMATE algorithm, would be a real asset and would lead to a very complete study.

Reply5: We appreciate this valuable suggestion. It would be more meaningful and complete if other components in GBM microenvironment such as endothelial cells could be deeply investigated. Despite the significant roles of endothelial cells in radioresponse, it is difficult to evaluate tumor vascularization with the current methodology. The ESTIMATE algorithm is used to infer the level of infiltrating stromal and immune cells in tumor tissues based on expression data. The stromal and immune scores can be obtained for each sample across all TCGA tumor types. A growing body of research has identified prognostic genes related to microenvironment by calculating immune and stromal scores using ESTIMATE algorithm in many cancers. However, as far as we know, the quantification of tumor vascularization is mainly based on immunohistochemical techniques in vitro or imaging system in vivo, which may not apply to this study.

Comment6: Overall, the font of the writings in the figures and the supplementary figures is small and therefore leads to figures that are difficult to correctly read.

Reply6: Thank you for the valuable advice. We have enlarged the font of the writings in the figures and the supplementary figures. We also increased the resolution of the images for better reading.

Changes in the text6: We modified the figures and supplementary figures according to the comment.

Minor points:

Comment1: A more suitable title would be appreciated, in particular by adding the notion of microenvironment or of immune and stromal parts (line 4).

Reply1: Thank you for the title suggested. After rigorous consideration and discussion, we believe the current title can concisely illustrate the basic idea of the manuscript. Thus, it may be appropriate to keep this one.

Comment2: In the abstract (background section), it would be appreciated to define microenvironment by adding the concepts of the immune and stromal compartments. It should be recited in the material and methods section if the patents included in this study have de novo or secondary GBM, or if these 2 types of GBM are mixed (line 46). Moreover, it would be pertinent to cite 10 genes identified with prognostic value and involved in GBM radioresistance (line 58).

Reply2: Thank you for the suggestion. According to the comments, the

precedent version of the abstract has been replaced. In the background section, we added one sentence to define GMB microenvironment: Immune and stromal cells are the two major types of non-tumor cells in the glioblastoma (GBM) microenvironment, which play critical roles in the prognostic assessment of tumors. In the material and methods section, we added "primary" before GMB to confirm the type of our patient cohort. In the result section, we cited 10 genes with prognostic value and involved in radioresistance.

Changes in the text2: We have modified the abstract as advised (see Page4, Line47-49; Page4, Line53; Page5, Line67-68)

Comment3: In the introduction section, the sentence at the line 67 is not correct: GBM is the most common primary brain tumor in adults. Likewise, the definition of the microenvironment is not sufficiently exhaustive (lines 82 to 85): the infiltrating immune cells also include lymphocytes, neutrophils and dendritic cells. In addition to immune cells and stromal cells, endothelial cells have a major role in the microenvironment.

Reply3: We appreciate the comment. According to the comments, the introduction section has been revised. We corrected the sentence as: Glioblastoma (GBM) is the most common primary brain tumor in adults and has a high rate of mortality. We also modified the define of microenvironment as: The GBM microenvironment contains a diverse

array of non-tumor cells, including immune cells, stromal cells, and endothelial cells, as well as extracellular matrix components. The two major types of cells in the GBM microenvironment are infiltrating immune cells (such as microglia, macrophages, lymphocytes, neutrophils and dendritic cells) and stromal cells (such as neurons, astrocytes, and oligodendroglia). However, we did not introduce endothelial cells in detail because the concept of tumor vasculature was less relevant to this study and more details about endothelial cells seemed to be redundant.

Changes in the text3: We modified some sentences as advised (see Page6, Line73; Page6-7, Line89-93).

Comment4: The authors used MDAR reporting checking list (line 109) but the TCR journal recommends to prepare the original articles according to the EQUATOR research reporting guidelines. I seem that MDAR reporting is not listed in EQUATOR reporting guidelines. Can the authors justify this choice and give the MDAR abbreviation?

Reply4: Thank you for the suggestion. MDAR (Materials Design Analysis Reporting) reporting checking list was required by the editor in the first-round review.

Comment5: Add reference at the end of this sentence "Although Jia et al. identified genes with prognostic value in the GBM microenvironment, the

relationship between microenvironment-related genes and radioresistance of GBM is unclear." (lines 96 to 98).

Reply5: Thank you for your comment. We have added this reference at the end of this sentence.

Changes in the text5: We have added this reference at the end of this sentence (see Page7, Line107).

Comment6: In the material and methods section, although a bibliographic reference illustrates the ImmuneScore, Stromal Score and ESTIMATEScore, it would be appreciated to briefly describe the methodology to obtain them, including listing the gene expression signatures used (lines 118 to 123).

Reply6: Thank you for the suggestion. As mentioned in the material and methods section, the Immune Score and Stromal Score of TCGA GBM cases were just downloaded from a public source website ***https://bioinformatics.mdanderson.org/estimate/,*** which can be easily accessed and need no specific description.

Comment7: At the lines 126 and 127, it is noted "The 348 tumor cases from the TCGA GBM database were assigned high or low immune 126and stromal scores relative to the median ImmuneScore and StromalScore, respectively." Could be give the median values for ImmuneScore and

StromalScore? What about you the ESTIMATEscore quoted in the line 121?

Reply7: Thank you for the comment. The median values for ImmuneScore and StromalScore have been added in the text. The ESTIMATEscore represents the purity of tumors and is not used for evaluating the prognosis and radioresponse of GBM patients.

Changes in the text7: We added the median values in the text as suggested (see Page11, Line194-195).

Comment8: In the result section, give the abbreviations of GO, BP, MF and CC used the first time in the article (lines 203-204)?

Reply8: Thank you for the suggestion. The abbreviations of GO, BP, MF and CC have mentioned in the material and methods section for the first time in this article. Thus, all the terms were shown in abbreviations in the result section.

Comment9: The Supplementary Figure 3 is not cited in the text of results. It would be pertinent to include this figure directly in Figure 2.

Reply9: Thank you for pointing out the mistake. The Supplementary Figure 3 has been cited in the text.

Changes in the text9: We added the cite as suggestion (see Page13, Line218).

Comment10: In the discussion part, in addition to describing the involvement of stromal cells in GB radioresistance, it would be interesting to do the same with immune cells (lines 251-252). Moreover, the sentence at the lines 259 to 261 could be revised because Jia et al. (reference 8) demonstrated that overall survival of GB patients was significantly correlated but not the stromal score.

Reply10: Thank you for the advice. We added one sentence to describe the involvement of immune cells in radioresistance: With respect to immune cells, macrophages and microglia in the microenvironment induce stemness and chemo-radioresistance in GBM cells.

Jia et al. showed the median overall survival of cases with the low score group of immune or stromal scores was longer than the cases in the high score group but it was not statistically significant. Therefore, we claimed that "a previous study reported no significant correlation between OS and the immune and stromal scores of patients with GBM".

Changes in the text10: We added one sentence according to the comment (see Page15, Line268-269).

Comment11: Concerning the discussion on CD163, I think it is necessary to specify that it is a specific marker of M2 macrophages in GBM (Vidyarthi A et al. Predominance of M2 macrophages in gliomas leads to the suppression of local and systemic immunity. Cancer Immunol

Immunother. 2019;68(12):1995-2004), which are known to be involved in the radioresistance of these brain tumors (Leblond MM et al. M2 macrophages are more resistant than M1 macrophages following radiation therapy in the context of glioblastoma. Oncotarget. 2017;8(42):72597-72612).

Reply11: Thank you for the comment. This paragraph was revised and modified according to the suggestion by the reviewer.

Changes in the text11: we modified the paragraph as suggested (see Page16-17, Line301-302).

Reviewer B

Comment1: In Figure 1A-B and Figure 4A-H, can the author provide information on the mean/median overall survival? Despite the statistical significance of the data, the biological significance appears inconsequential (ie: Fig 4A, 4B).

Reply1: Thank you for the comment. All the median overall survival has been added in the Figure1A-B and Figure 4A-H.

Changes in the text1: We added the median overall survival in the mentioned figures (see Figure1A-B and Figure 4A-H).

Comment2: Has the author performed GSEA statistical analysis to confirm the significance of the enrichment analysis as shown in Figure 2?

Reply2: Thank you for the comment. We utilized the GSEA online tool (***https://www.gsea-msigdb.org/gsea/index.jsp***) to confirm the significance of the enrichment analysis as shown in Figure 2. The key signaling pathways listed in the Figure2 and supplement table 1-4 were also significant in GSEA statistical analysis.

Comment3: In figure 3, the author performed string analysis to determine protein-protein interaction (PPI) with a cut-off of 0.4 (medium confidence). Will the PPI remain identical when a 0.7 (high confidence) cut-off is applied to the analysis and how does that affect the overall conclusion of the data? Additionally, the author should further elaborate in the discussion how each network is potentially associated with the properties of radioresistance.

Reply3: Thank you for the comment. We used a cut-off of 0.4 (a default value) to avoid negative readout. According to your suggestion, we set the cut-off of 0.7 as high confidence. The results showed that the network included 104 nodes and 440 edges and consisted of four significant modules according to the results of the Molecular Complex Detection method. The three representative nodes with a cut-off of 0.4 were still included in the network with the cut-off of 0.7. The relationship between the whole network and the properties of radioresistance has been less reported. However, we found some related DEGs in the network were

associated with radioresistance which were mentioned in the discussion, such as CD163, NCF2, TLR2.

Comment4: It will be interesting to know if 10 of the 19 DEGs that were identified by the author is druggable. The author should consider a quick analysis using tools such as connectivity map (CMAP) to strengthen the manuscript in relation to novel therapeutics.

Reply4: We appreciate for the interesting suggestion. As far as we know, there are no targeted drugs for these DEGs up to now. We think it would be instructive to study on these selected DEGs which will provide further insights into novel therapeutics. We believe the selected DEGs are potential biomarkers that would be targeted and applied for novel therapeutics in GBM management in the near future.