Peer Review File

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Reviewer A

The manuscript "Targeting complement C5a to improve radiotherapy sensitivity in non-small cell lung cancer" by Yuan et al., is proposed to study the role of the complement activation in the tumor microenvironment following irradiation.
Mice were inoculated subcutaneously with mouse lung cancer cells (Lewis lung carcinoma) and then irradiated with 8 Gy of x-rays.

1) the infiltration of CD8+ T cell was measured and RNA of RT-recruited CD8+ T cells was sequenced.
2) tumor growth was measured +/- C5aR1 inhibitor.
3) expression of C5a/C5aR1 and their signaling pathways was studied on irradiated tumor tissues and tumor cells.

The authors observed that irradiation induced a CD8+ T cells infiltration and a local activation of complement C5a/C5aR.
C5aR inhibitor improved radiosensitivity and tumor-specific immune response.
The AKT/NF-κB pathway was found to be an important signaling pathway in C5a/C5aR axis mediation by RT.

The authors concluded that C5aR inhibition could improve radiotherapy of lung cancer.

The strategy of the author is very well defined, and the results are well organized and presented.

Reply: Thank you for your comments concerning our manuscript. We quite appreciate your insightful comments. The comments are valuable and very helpful for revising and improving our paper.

Reviewer B

The paper titled "Targeting complement C5a to improve radiotherapy sensitivity in non-small cell lung cancer" is interesting. The work provides evidence that the combination of RT and C5aR blockade opens a new window of opportunity to promote anti-tumor therapeutic effects in lung cancer. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.
Reply 1: Thank you for your comments concerning our manuscript. We have modified our text as advised (See Page 4, line100-101, Page 5, line122-123 and Page 5, line143-144)
Changes in the text: Page 4, line100-101, Page 5, line122-123 and Page 5, line143-144.

Comment 2: In the results of this study, it was mentioned that "This phenomenon was further confirmed in IF staging and flow cytometry (Figure 1C)". But the figure shows that the IF staging is shown in Figure 1C, while the flow cytometry result is shown in Figure 1D. Please carefully check and make corrections.
Reply 2: Thank you for your comments concerning our manuscript. We have modified our text as advised (See Page 10, line 313)
Changes in the text: Page 10, line 313.

Comment 3: How to identify and verify the radiation sensitivity characteristics of patients with NSCLC? It is recommended to add relevant content.
Reply 3: We used an LLC tumor bearing mouse model and an LLC cell line to investigate RT sensitivity. We recognize the limitations of using animal models to simulate human clinical diseases. Conclusions based on animal experiments needed to be validated by further clinical studies. We will add clinical patient data to prove our conclusion in the following studies.

Comment 4: There are many genes that regulate the radiotherapy sensitivity in NSCLC. Why did the author choose complement C5a for research? Please describe the reason.
Reply 4: Firstly, the combined antitumor effects of RT combined with inhibitation of complement system have been found. Secondly, C5a/C5aR axis has been clarified to improve antitumor effect of chemotherapy and immunotherapy. Thirdly, we found that RT mediated functional pathways exist C5aR1 gene enrichment. It was also found that expression of the C5aR1 gene in CD8+ T cells increased significantly after RT. Last, C5aR is the specific receptor of C5a and RT promotes the release of C5a from tumor cells in our study. Thus, we choose complement C5a to research the radiotherapy sensitivity in NSCLC.

Comment 5: Figures 2 and 3 are not clear enough. It is recommended to provide clearer figures again.
Reply 5: Thank you for your sincere suggestion. We made some changes to these figures to make it easier to read clearly. In addition, we have confirmed with the editor that the size of these figures is adequate in a printed version.

Comment 6: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "LncRNA KCNQ1OT1 enhances the radio resistance of lung squamous cell carcinoma by targeting the miR-491-5p/TPX2-RNF2 axis, J Thorac Dis, PMID: 36389338". It is recommended to quote the articles.
Reply 6: Thank you for your sincere suggestion. We have modified our text as advised (See Page 4, line100-101)
Changes in the text: Page 4, line100-101.

Comment 7: What is the impact of this study on the further treatment and prognosis of NSCLC? It is recommended to include relevant content in the discussion.
Reply 7: Thank you for your sincere suggestion. We have modified our text as advised (See Page15, line486-489)
Changes in the text: Page15, line486-489.

Reviewer C

1. Mice/mouse and rat(s)

As mice/mouse and rat(s) are not the same kind of animal, please double check and unified the expressions in the manuscript.

169 ##*In vivo* experiment models

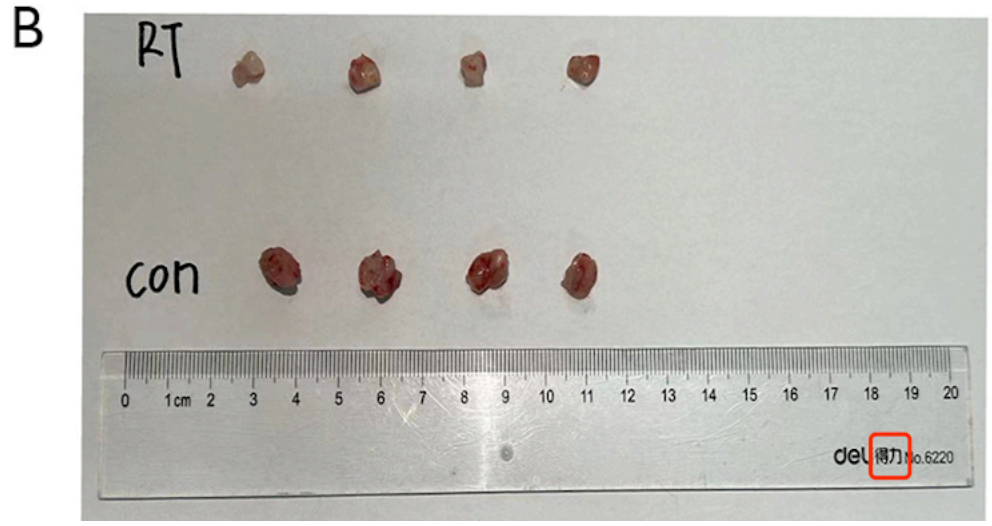
170 To explore the antitumor effects of RT and blockade of C5aR signaling, 1×10^6 LLC

171 cells were subcutaneously injected into the right hindlimb of experimental rats. The

Reply: Thank you for your sincere suggestion. We have modified our text as advised. (See Page 6, line 171)

2. Figure 1

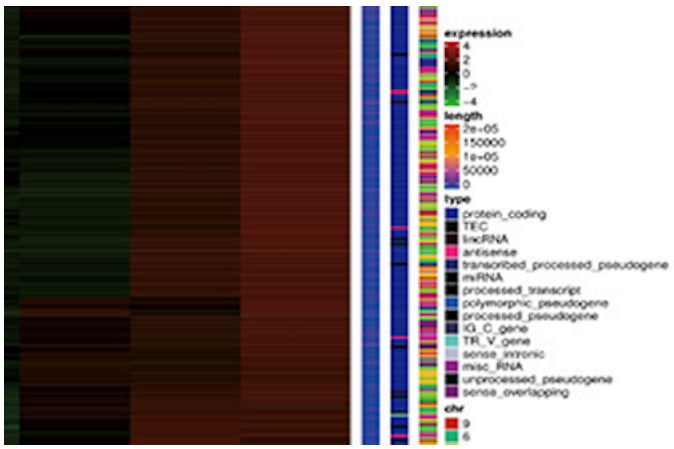
Please remove the Chinese in the figure..



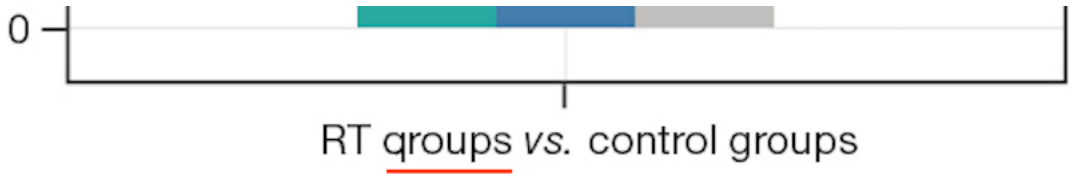
Reply: Thank you for your sincere suggestion. We have modified our text as advised.

3. Figure 2

a) Please provide a clearer version of 2B, the current one cannot be seen clearly (as you can see the screenshot below).



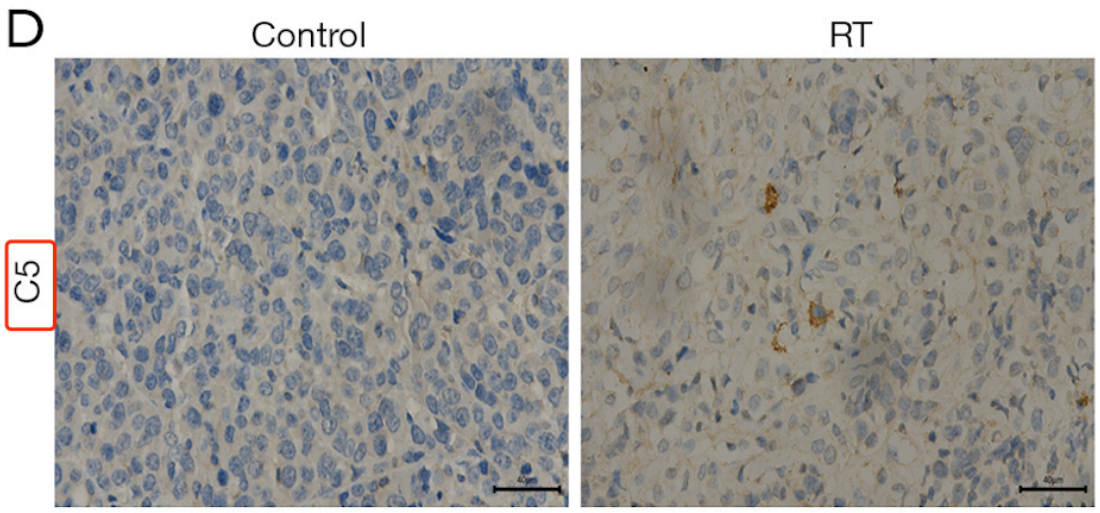
b) In 2C, the x-axis should be RT groups, please revise.



Reply: Thank you for your sincere suggestion. We have modified our text as advised.

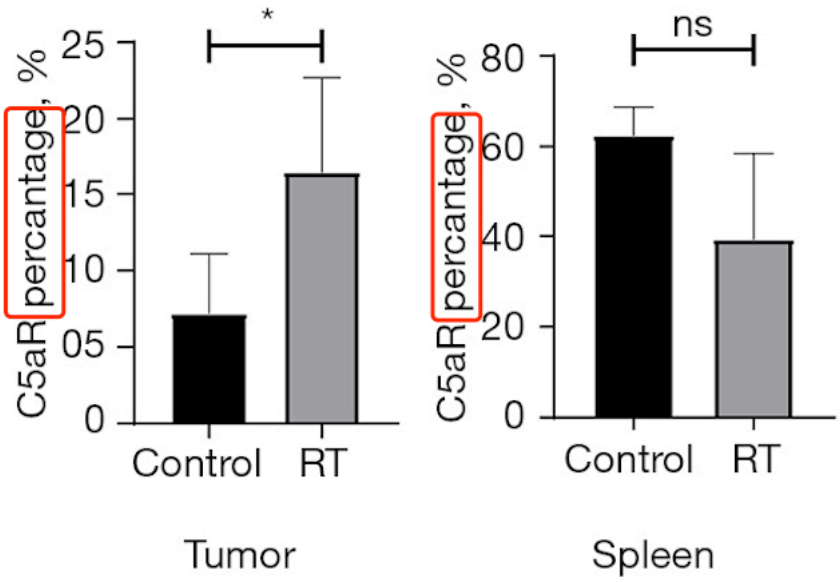
4. Figure 4

a) Please check which is correct, C5 or C5a?



742 (B) and spleen tissues (C). (D) Representative images of C5a staining in tumor tissues
743 in irradiated and untreated groups. Scale bars represent 40 μm. (E) IF of tumors in

b) Here should be percentage, please revise. Besides, please remove the % in the y-axis, it is the same as the percentage, just keep one is enough.



Reply: Thank you for your sincere suggestion. We have modified our text as advised.

5. References/Citations

Please double-check if more studies should be cited as you mentioned “studies”.

382 had already been characterized (37). Interestingly, studies had shown that the activation
383 of the PI3K/AKT pathway might be a latent mechanism of therapeutic resistance of
384 small cell lung cancer (SCLC) (38). Consequently, we hypothesized that RT might

Reply: Thank you for your sincere suggestion. We have modified our text as advised. (See Page 12, line 382-384)