List of Responses

Dear Editors and Reviewers：

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled"". those comments are all valuable and helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewers' comments are as follows:

Responds to the reviewer's comments:

Reviewer #1:

1. The section of patient demographics in Results should be shown in M&M, and "Expression patterns of RON and PD-L1 in the GEO cohort" should be described in detail.

Response: Thanks for your careful checks. It is really true as Reviewer suggested that the patient demographics in Results show in M&M. We have adjusted the position of this section to pages 8 (from lines 176 to 185) and marked in red.

And we are very sorry for our negligence of described this part in detail. We have added this explanation in the "Expression patterns of RON and PD-L1 in the GEO cohort" in page 14 (from lines 326 to 330) and marked in red.

2. In general, authors should use more than 2 cell lines to validated their findings in in vitro experiments. I am puzzled why LoVo cells with lower toxicity was not used, which appeared in Supplemental.

Response: Thank you for your comment. In this study, we need a cell line that expresses both RON and PD-L1. We found that LoVo cells express PD-L1 and do not express RON by flow cytometry. So we only select HT29 in our vitro experiments.

3. It is unreasonable for authors to assess the relationship between RON and PD-L1 by a multiplex immunofluorescence staining. They should use quantitative assays such as RT-PCR and ELISA, etc.

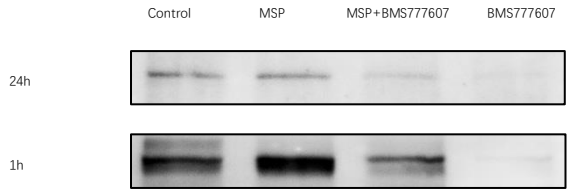
Response: Thank you for your reminding. In response to your suggestion, we have modified the language to make it more accurate on page 14 (lines 340) and marked in red. Multiple immunofluorescence staining can more completely and visually display the expression of RON and PD-L1 in the tumor microenvironment of the patient's tumor tissue. We believe that RT-PCR and ELISA experiments cannot better show their expression. Then we evaluated the expression levels of different colorectal cancer tissue samples using a semi-quantitative method called immunohistochemistry, and analyzed their correlation through the statistical method of chi-square test.

4. Fig 3c which should be replaced by new one is too difficult to distinguish;

Response: we are very sorry for present low-quality pictures in Fig 3c, we have reselected and replaced the images to make it more distinguishable.

5. The number of cells is too few in Fig 5a; Phosphorylated RON should also be detected in Fig 5b. The picture quality of PD-L1 in Fig 5b should be improved.

Response: we are very sorry for present low-quality pictures in Fig 5, we have replaced this with higher quality pictures. Normally, the phosphorylation of RON receptor tyrosine kinase occurs in a short period of time and reaches a peak in 1-2 hours. In articles related to RON, researchers usually only detect the phosphorylation of RON receptor tyrosine kinase for 1 hour[1]. After 24 hours, the phosphorylation state of RON is unstable. Our results also show that phosphorylation of RON is unstable at 24 hours.



1. O'Toole, J.M., K.E. Rabenau, K. Burns, et al., Therapeutic implications of a human neutralizing antibody to the macrophage-stimulating protein receptor tyrosine kinase (RON), a c-MET family member. Cancer Res, 2006. **66**: 9162-70.[PMID:16982759 doi:

10.1158/0008-5472.]

6. Authors should supply with some information and intention in this study of inhibitor MSP.

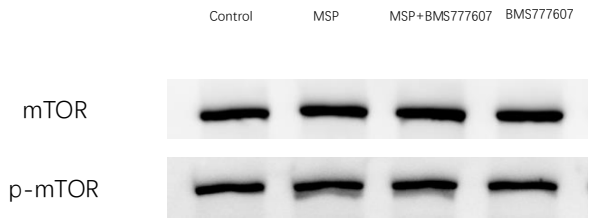
Response: Thank you for your comments. We have added this information and intention in page 9 (from lines 193 to 196) and marked in red.

Reviewer #2:

The article with the title "The pathological significance of abnormal RON and PD-L1 expression in colorectal cancer" is in generally well done, but I would offer these comments to the investigators:

1. **Figure 6. I highly recommend to check also downstream agents of AKT such as mTOR, ps6r etc.**

Response: Thank you for your comments. I followed your suggestion to detect the AKT signaling pathway downstream of mTOR protein by western blot, and found that the phosphorylation of RON did not affect the protein level of mTOR.



2. **i) Figure 6 A. The authors identified the activation of the signaling pathways MEK/ERK and AKT/mTOR. Both pathways are regulators of autophagy, a significant mechanism for several cellular processes including the emerging role of autophagy in shaping the crosstalk between the cancer cells and the tumor microenvironment and PD-L1 expression in cancer cells. I recommend the authors to perform an experiment in order to identify the levels of autophagy markers such as LC3II, p62, Beclin-1. You can discuss the possible correlation between authophagy activation and PD-L1 expression. Koustas E, et al. PLoS One. 2018;13(11):e0207227. doi: 10.1371/journal.pone.0207227. Koustas E, et al. Cancers (Basel). 2019;11(4):533 doi: 10.3390/cancers11040533. ii) If autophagy is activated, an autophagy inhibitor such as Hydroxychloroquine will reveal a possible mechanism of action for your model.**

Response: Thank you for your suggestions. Our experiments proved that the activation of RON did not enhance the expression of mTOR, which is the key to autophagy.

Therefore, the level of the corresponding autophagy marker protein downstream of mTOR may not be changed, such as LC3II, P62, beclin-1. In this study, we mainly discussed the expression of RON and its activation related pathways, and then studied the effect of RON on the expression of PD-L1 in colorectal cancer tissues. This is different from the autophagy mechanism, and the two studies focus on different aspects of the expression of PD-L1. However, we discuss it on pages 19 (from lines 477 to 781) in the article based on the literature you provided and marked in red.

3. **It is properly to mention that the antibody against p-AKT detects the endogenous levels of Akt only when phosphorylated at Ser473. Because the complicate mechanisms of PI3K/AKT/mTOR axis activation with the positive feedback loop (AKTthr308, ser473, mTOR1 and mTOR2) on autophagy, I believe that it will be useful to discuss it.**

Response: We feel great thanks for your professional review work on your article. Our experiments showed that RON phosphorylation did not affect p-mTOR protein levels. We discuss it on pages 19 (from lines 477 to 781) in the article based on the literature you provided.

4. **All sections need to be revised to take into account all revisions made.**

Response: Thank you for your comment. I have supplemented, discussed and explained all your suggestions.

Editor Comments

(1) ***Science Editor*****:** 1 Scientific quality: The manuscript describes a basic study of the RON and PD-L1 expression in colorectal cancer. The topic is within the scope of the WJG.

(1) Classification: Grade B and Grade C;

(2) Summary of the Peer-Review Report: The study is interesting, and the manuscript is well written. However, there are some issues should be addressed. It is unreasonable for authors to assess the relationship between RON and PD-L1 by a multiplex immunofluorescence staining. Authors should supply with some information and intention in this study of inhibitor MSP. You can discuss the possible correlation between autophagy activation and PD-L1 expression. The questions raised by the reviewers should be Responded; and (3) Format: There are 3 tables and 6 figures. A total of 50 references are cited, including 13 references published in the last 3 years. There are no self-citations.

2 Language evaluation: Classification: Grade B and Grade B. A language editing certificate issued by Elixigen was provided.

3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and the Institutional Review Board Approval Form. No animals are involved in the study. No academic misconduct was found in the CrossCheck detection and Bing search.

4 Supplementary comments: This is an invited manuscript. The study was supported by Zhejiang Provincial Natural Science Foundation of China grant; National Natural Science Foundation of China grant; and the Zhejiang Major Medical Health & Sciences Technology Foundation Projects. The topic has not previously been published in the WJG. The corresponding author has not published articles in the BPG.

5 Issues raised:

(1)I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

Response: we are very sorry for did not provide the approved grant application form(s). We have upload corresponding document in the attachment.

(2) I found the authors did not provided the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Response: we are very sorry for missing this part, we have provided a PowerPoint including the original figures in the attachment.

(3) I found the authors did not write the "article highlight" section. Please write the "article highlights" section at the end of the main text; and

Response: we are very sorry for our negligence, we have added the corresponding content on page 21-23 (from line 527 to 578) and made it conform to the language specification and content requirements.

(4) co-corresponding author is not allowed.

Response: Thank you for your suggestions. We have listed only one corresponding author according to your request.