Peer Review File

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Reviewer A

The paper titled "RRP12 suppresses cell migration and invasion in colorectal cancer cell via regulation of epithelial-mesenchymal transition" is interesting. RRP12 is involved in the tumorigenesis and metastasis of CRC by regulating the EMT process through ZEB1. Thus, RRP12 could be a potential therapeutic target for CRC therapy. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the potential relationships between RRP12, epithelial-mesenchymal transition and cancer stem cells? How interaction of these processes may affect colorectal cancer progression, chemoresistance and ultimately recurrence? It is recommended to add relevant content.

Reply：Recommended contents have been added in the introduction part of this text.

Changes in the text: line 77-80

2) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply：modification have been made in the introduction part of this text.

Changes in the text: line 29-31

3) What is the correlation between the expression of RRP12 and the prognosis of colorectal cancer patients? How to gain in-depth understanding through bioinformatics?

Reply：RRP12 is highly expressed in CRC cell lines and clinical samples and is associated with poor survival in CRC patients. Biogenic analysis suggested that high expression of RRP12 was associated with poor prognosis of CRC.

Changes in the text: line 43-44，49-50

4) There are many genes that regulate the colorectal cancer. Why did the author choose RRP12 for research? Please describe the reason.

Reply：We found that the expression of RRP12 is associated with the prognosis of CRC through biogenic analysis, but the possible mechanism is less studied.

Changes in the text: line 78-87

5) All figures are not clear enough. It is recommended to provide clearer figures again.

Reply: All of the figures have a resolution of 300bpi.

Changes in the text: None

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "The miRNA125a-5p and miRNA125b-1-5p cluster induces cell invasion by down-regulating DDB2-reduced epithelial-to-mesenchymal transition (EMT) in

colorectal cancer, J Gastrointest Oncol, PMID: 36636074". It is recommended to quote the articles.
Reply: Recommended article has been cited in the introduction part of this paper.
Changes in the text: Line 496-499.

7) What is the correlation between RRP12 and the tumor microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.
Reply：Our current study did not focus on the correlation between RRP12 and the tumor microenvironment, and relevant trials will be conducted in the future to make it clear.
Changes in the text: None

Reviewer B

Submitted study corresponds to the current trends in cancer research, as searching for new biomarkers of colorectal cancer is relevant topic in the field. Although the paper reports some new interesting observation I found the paper is not clearly written and, because of some data missing, with overestimated conclusions. I have too many questions and doubts to be able to recommend this paper for publication without major revision.

Major comments:
1. Checklists are not properly filled and don't answer the recommendations.
Reply: We refilled the checklists as required.
Changes in the text: None

2. The authors stated that RRP12 is highly expressed in 4 studied CRC cell lines, which serves as basis for further analyses. Unfortunately, they haven't shown it. The representative western blot with the graph showing densitometry of blots from all replicates of experiment is necessary for showing this result and conclusion. Otherwise, it may be speculative. The result form RT-PCR is not enough.
Reply：Figure1D showed RRP12 expression were detected in different types of CRC cells.
Changes in the text: None

3. What was the reason of taking into consideration HCT116, RKO, SW480, LoVo to the research? It should be also explained why for next steps only HCT116, SW480 were chosen.
Reply: Because these four cell lines are commonly used colorectal adenocarcinoma cell lines, and RRP12 is highly expressed in HCT116 and SW480.
Changes in the text: None

4. Could the authors explain why they performed the silencing only in HTC116 cell line and the up-regulation only in SW480 instead of performing both in both cell lines? As we can get different results from different cell lines, the result shown by the authors in such way as here can't be generalized. So, also the results form in vivo can' t be compared between cell lines and be generalized.

Reply：Because RRP12 is highly expressed in both HCT116 and SW480 cell lines, as to verify the general effect of RRP12 expression in different colon cancer cell lines, we chose to knockdown or overexpress it in different cell lines. We agree with your point of view, and will supplement relevant content in subsequent experiments, and carry out knockdown and overexpression experiments in the same cell line.
Changes in the text: None

5. For conclusions according to silencing and up-regulation of RRP12 are shown it would be valuable to show on the graph also non-transfected cells (untreated cells) , as control, in each experiment. Also when Western Blot study of EMT markers and ZEB-1, unstimulated cells are essential.
Reply： We agree with your point of view, and will supplement relevant content in subsequent experiments.
Changes in the text: None

6. All experiments were done in triplicate, so , besides the representative blots in each Western blot experiment, the graph showing densitometry analysis is needed to be shown to make the conclusions. Otherwise the conclusions are overstated.
Reply： We strongly agree with your modification opinion and will conduct statistical analysis of the gray value of the Western Blot results in the subsequent work.
Changes in the text: None

7. According to my knowledge the proliferation of cells should be investigated between 48 and 96 h form the beginning of experiment. Proliferation at 24h is too short. So the authors should properly design this assay and add the results obtained after longer times to get the result and conclusion.
Reply：We agree with your point of view, and modification have been made in the text.
Changes in the text: line 181

8. What did the authors mean when they wrote: RRP12 knockdown was found to reverse metastasis of CRC cells in vivo.? Have the authors really shown in the in vivo experiment that metastasis was reversable?
Reply：In vivo experiments, RRP12 knockdown can reverse metastasis of colon cancer cells by comparing changes in lung metastatic lesion size and EMT-related indicators.
Changes in the text: None

9. It is hard to figure out how some experiments were designed- neither form methods nor from results. For example in vivo experiment.
Reply： The methods and results of in vivo experiments have been revised as required.
Changes in the text: Line 185-191

10. Some sentences are not precisely written and they are confusing, for example “3 pairs of non-metastatic and metastatic CRC tissue specimens were collected”, “metastatic desease”.

Reply： Non-metastatic CRC means that the tumor has not metastasized far away.
Changes in the text: None

11. The legends and titles of figures also should be carefully redrafted as they are not precise and misleading.
Reply：All of the figures have a resolution of 300bpi.
Changes in the text: None

12. It is hard to figure out what is presented on some figures due to lack of titles of graphs/pictures (e.g. cell line names, titles of assays, P1-3, N, M).
Reply：The titles of the pictures and tables are displayed in the corresponding positions in the text.
Changes in the text: None

13. There is a mess in the description of methods and of results and legend of fig. as far as migrative phenotype of CRC cell lines. The authors should precisely inform what is the difference between migration and invasion. And also between motility and migration. Using words as "respectively" introduces confusion while reading some parts of the text.
Reply: We agree with your point of view, and will make corresponding modifications in the text.
Changes in the text: Line 164-173

14. The authors use "motility" when showing the results from wound healing method. This method is other method to show the migration of the cells. The use of the "motility" word in manuscript should be rethought.
Reply：We agree with your point of view, and corresponding modifications have been made in the article.
Changes in the text: Line 528-529

15. Wound healing assay is not described in methods at all.
Reply：We agree with your point of view, and have added Wound healing assay to the methods section of the text.
Changes in the text: Line 157-161

16. Additionally to fig 5, the authors should provide, as supplementary data, full different characteristics between low- and high-RRP12 groups in TCGA and GEO.
Reply：We agree with your point of view，if required, we can provide the above result as supplementary data.
Changes in the text: None

17. Method section should be carefully redrafted. Many important information is missing or the info is written in such way that the reader can't understand the meaning: lines 120-122.

Details of databases, names of manufacturers, concentrations of the factors, incubation time post-delivery before particular experiments, transwell membranes pore size, concentration and volume of Matrigel, etc.

Reply：We agree with your point of view, and corresponding modifications have been made in the text.

Changes in the text: Line 124-125

18. The information about approvals are too general.

Reply：We have checked the ethical approval documents of the relevant research in the article, and the study concerning human specimens and animal experiments were approved by the Ethics Committee of our hospital respectively and expressed in the corresponding parts of the article.

Changes in the text: Line 110-111，Line 188-191

Minor problems:

1. There are a lot of uncertainties in the text which must be clarified, e.g. “patients were pathologically diagnosed with CRC”- what does it mean? Some details, for example CRC stage should be added. “242 paraffin blocks”- from how many patients?

Reply：All patients were pathologically diagnosed with colorectal cancer (CRC); “242 paraffin blocks” were from 242 patients; the TNM staging is described in Table 2 of the results.

Changes in the text: None

2. Why in in vivo experiment only lungs were stained? What about liver metastases which are known to be common in CRC?

Reply：We will carry out relevant experiments on liver metastasis specimen in vivo experiment subsequently.

Changes in the text: None

3. In fig 3 the result from luciferase assay is shown while this assay is mentioned neither in methods nor in the results.

Reply：Figure 3 shows the results of lentivirus transfection of cells, not luciferase assay.

Changes in the text: None

4. The authors can omit the abbreviation of DNA in Abstract and in manuscript.

Reply：We have removed the full name of DNA in the abstract and manuscript.

Changes in the text: Line 34

5. Sometimes the authors made mistakes in the text, e.g. breast cells, b-actin as a group, cells instead of cell lines.

Reply：Thank you for your reminding, we have made corresponding modifications in the manuscript.

Changes in the text: Line 525-526

I am afraid that checklists (The ARRIVE Essential 10 and The Recommended Set) are filled not in accordance with the line numbers of manuscript. Almost no line number given by the authors is correct and answers the recommendation. It looks like randomly filled. MDAR needs to be completed because some details are missed. The authors don't provide any numbers for approvals (experimental animals, human research participants).

Reply：Thank you for your reminding, we have refilled the checklists as required.

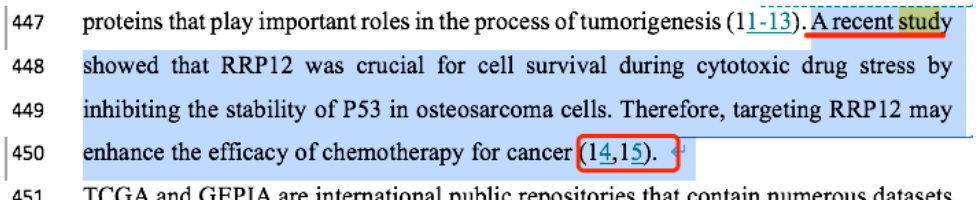
Reviewer C

1. **References**

a. References (10 and 15) are the same. Please delete one of them, and update the citation in both the main text and the reference list.

Reply: Thank you for your reminding, the modification has been made in the text.

b. "Recent studies" is more appropriate, as two studies cited here.


447 proteins that play important roles in the process of tumorigenesis (11-13). A recent study
448 showed that RRP12 was crucial for cell survival during cytotoxic drug stress by
449 inhibiting the stability of P53 in osteosarcoma cells. Therefore, targeting RRP12 may
450 enhance the efficacy of chemotherapy for cancer (14,15).
451 TCGA and GEPIA are international public repositories that contain numerous datasets

Reply: Line 354-355

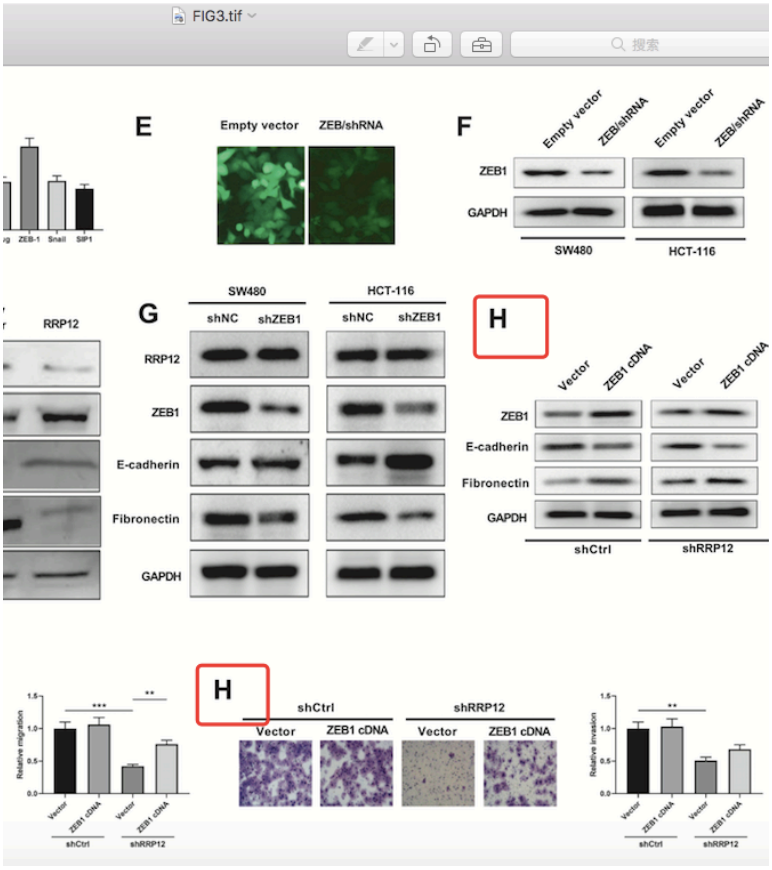
2. **Figure 2F, G and Figure 3I, J**

For cell map, please indicate the staining method in figure legends.

Reply: stained with 0.5% crystal violet in Transwell assay.

3. **Figure 3**

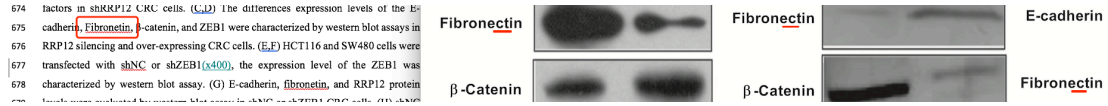
a. Two panels are marked as "H", please check.



Reply: Figure 3 has been revised.

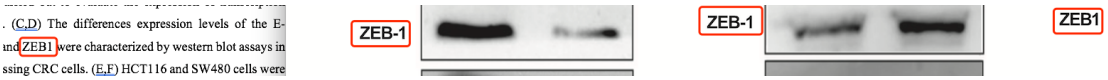
4. Figure 3C, D, G, H

a. please check which word is correct: Fibronetin or Fibronectin?



ReplyFibronectin is correct. Line 540.

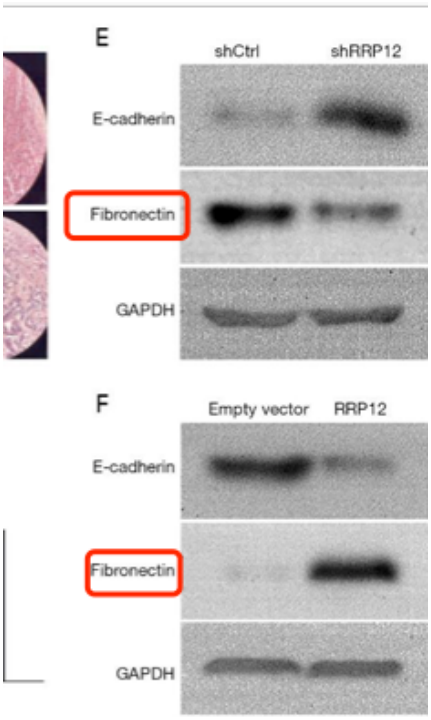
b. Please unify.



ReplyThe final version of Figure 3 has been revised in the text.

5. Figure 4E, F

Please check which word is correct: Fibronetin or Fibronectin?



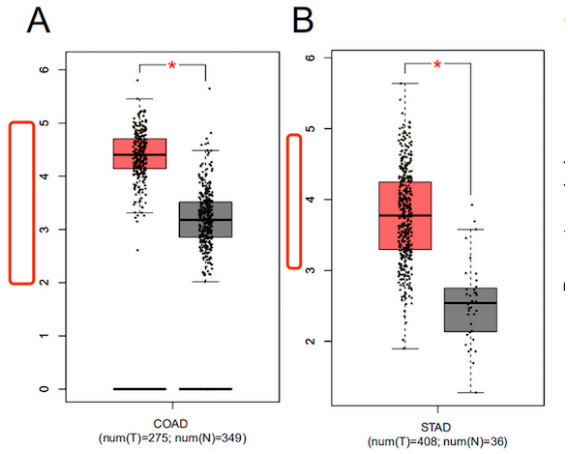
Reply：Fibronectin is correct.

6. Figure 5

a. Please add description (with measure unit, if applicable) for the Y-axis.

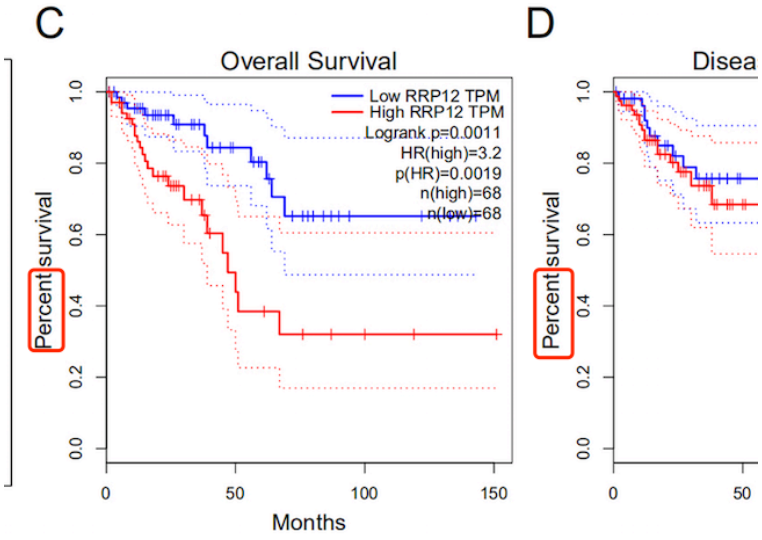
Reply: The modification has been made in Fig 5.

b. Please indicate the meaning of "*" in figure legend.



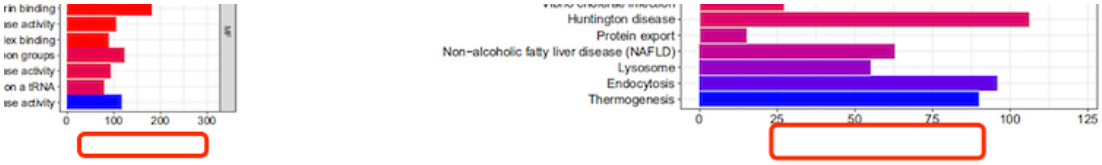
Reply: Line 568.

c. Please remove "percent", as the numbers are 0-1 in Y-axis.



Reply: Percent has been removed from the fig.

d. Please add description for the X-axis.



Reply: Not applicable.

e. Figure 5 is a (A-F) combined picture, but figure 5F is not cited in the main text. Figure 5F should be cited consecutively after Figure 5E. Please check.

Reply: Thank you for your reminding, the modification has been made in text. Line 336.