Author's response to reviews

Title: Expression of the phosphorylated MEK5 protein is associated with TNM staging of colorectal cancer

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Author's response to reviews: see over
Dear Dr. Upender Manne:

Thanks for inviting us to revise our manuscript (MS: 1561946464529928) entitled “Overexpression of the phosphorylated MEK5 protein is associated with the progression of colorectal cancer” submitted by Hu et al. to BMC cancer for publication.

In response to the comments from the editor and reviewers, we have revised the manuscript as follows: (a) we have modified the Title of our original manuscript. (b) We have rewritten the Abstract to make it more clear and read more smoothly. (c) We added the limitations of our study in Discussion. (d) We further stratified our cohorts by locations such as right colon cancer, left colon cancer, and rectal cancer. (e) We corrected two typographical errors of the manuscript.

We have revised the manuscript, according to the comments and suggestions of reviewers and editor, and responded, point by point, the comments as listed below. Since the paper has been revised significantly throughout the text, we feel it is better not to highlight the amendments in the revised manuscript.

We hope we have adequately addressed the reviewers’ concerns. We would like to thank you and the reviewers for all the efforts you have made to improve the quality of our manuscript. We appreciate the opportunity to have the revision to be reconsidered for publication in your journal.

Thank you and best regards.

Yours sincerely,

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Enclosures
1. Response to reviewers’ comments
2. Revised manuscripts
Response to reviewers’ comments on manuscript (MS: 1561946464529928) entitled “Overexpression of the phosphorylated MEK5 protein is associated with the progression of colorectal cancer” submitted by Hu et al. to *BMC cancer* for publication.

First of all, we thank both reviewers and editors for their constructive comments and suggestions which are definitely helpful to further improve our manuscript. We read the comments carefully and made correction which we hope can meet your requirements. Revised portions are marked in red. The main corrections in the manuscript and our responses to your comments are as following:

Reviewer 1:
Comments to the Author

The manuscript is well-written and well done. The findings and conclusions are of great interest to colorectal cancer prognosis and possibly therapeutic evaluation.

**Response:** Thank you very much for understanding merits of our study and providing very encouraging and positive comments.

Major concerns:

1. I find it interesting that the tumor location did not significantly correlate with the expression of pMEK5 while the tumor location was significantly associated with DFS and OS. While the authors did stratify location by colon and rectum, they could have further stratified location by right colon, left colon, and rectum since they all have developmental differences.

**Response:** Thank you for your comments. We agree that location stratification would be of great help to improve the quality of our manuscript. Our cohorts were further stratified
by right colon cancer, left colon cancer, and rectal cancer has been provided in Table 2 in the revised manuscript, however, we did not found significant difference. (Page 19, lines 10-13)

2. I agree that a larger series may prove worthy in better delineating the possible status of the protein as a biomarkers. Effects of neoadjuvant therapy on the expression of pMEK5 in rectal cancer and correlated prognosis would be interesting in follow-up studies.

Response: Thanks for your understanding and suggestions. We will continue to study the protein on more patients and treatment settings.

3. Two typos noted:

   Background: page 3, line 2 “actives” should be “activates”.

   Methods, page 4, 5th line from bottom “…patients underwent…” missing a word such as “…patients [that] underwent… ”.

Response: We appreciate you for carefully and patiently reviewing our manuscript. In the revised version, the corrections have been made as you suggested. (Page 4, line 2; and Page 5, line 18)

Reviewer: 2

Comments to the Author

The research question is clear. It is assessing the value of pMEK5 in assessing the prognosis of a CRC patient. The study seems well thought out. The analytical methods used are appropriate. The tables are well laid out. All in all the study is well done.
Response: Thank you very much for understanding merits of our study. We also appreciate you for carefully and patiently reviewing our manuscript and providing very encouraging and positive comments.

Major concerns:

1. The limitations of the work are not very clearly stated. This is a retrospective look at the expression of a biomarker and the study has all of the inevitable problems of the retrospective study of not being able to establish if the expression of the marker is after the progression has occurred. The value of the biomarker as an indicator of progression may thus be limited.

Response: Thank you for your careful review and comments. We agree that study limitation is important information that needs to be stated clearly. According to your comments, we added a section to state the limitations of our study in Discussion: “However, this study is retrospective look at the expression of pMEK5 in the CRC tissue, it is not able to establish if the expression of the pMEK5 is after CRC has occurred. And the mechanism of actions for pMEK5 protein is not known and needs further investigation” (Page 13, lines 19-22)

2. The abstract does not discuss the survival analysis at all. The authors thus do not describe the non-significance of pMEK5 in the multivariate Cox’s PHREG. The title also seems a little misleading given the non-significance in multivariate regression. The point the authors make in the discussion about the usefulness of pmek5 as a marker of TNM
stage is a good one and perhaps would be more appropriate to add in the title.

**Response:** Thank you very much for your suggestion and we agree with your suggestion. Accordingly, in the revised manuscript, the **Title** has been changed to “Expression of the phosphorylated MEK5 protein is associated with TNM staging of colorectal cancer”. And we have added some discussion of survival analysis in **Abstract:** “Analysis of the survival curves showed a significantly worse 5-year disease-free ($P=0.002$) and 5-year overall survival rate ($P<0.001$) for patients whose tumors overexpressed pMEK5. However, in multivariate analysis, pMEK5 was not an independent prognostic factor for CRC ($P=0.139$; OS: $P=0.071$)” (Page 2, line 20 - Page 3, line 2).

**Also,** we made additional corrections:

We have rewritten the **Methods** in **Abstract** to “pMEK5 expression was examined by immunohistochemistry in a tissue microarray (TMA) containing 335 clinicopathologic characterized CRC cases and 80 cases of nontumor colorectal tissues. pMEK5 expression of 19 cases of primary CRC lesions and paired with normal mucosa was examined by Western blotting. The relationship between pMEK5 expression in CRC and clinicopathologic parameters, and the association of pMEK5 expression with CRC survival were analyzed respectively” to make it more clear and read more smoothly. (Page 2, lines 6-12)

We have rewritten the **Conclusions** in **Abstract** to “pMEK5 expression is correlated with the staging of CRC and its expression might be helpful to the TNM staging system of CRC” to make it more clear and read more smoothly. (Page 3, lines 3-4)
We added a data “(P>0.05; Table 2)” in Page 9, line 19.

We deleted the sentence “Furthermore, pMEK5 may be a useful marker for metastasis of CRC” in the end of the original manuscript.