Dear Dr. Ma and reviewers,

Thank you very much for giving us an opportunity to revise the manuscript " Protein and gene expression characteristics of heterogeneous nuclear ribonucleoprotein H1 in esophageal squamous cell carcinoma" (ID: 25957). We revised the manuscript in accordance with the reviewer's comments, and carefully re-formatted the manuscript according to the Guidelines and Requirements for Manuscript Revision-Basic Study.

We would like to express our sincere thanks to the reviewers for the constructive and positive comments. Here below is our description on revision according to the reviewer's comments.

Reviewer: 1

This manuscript by Sun et al clearly demonstrated overexpression of HNRNPH1 in human esophageal squamous cell carcinoma and its association with poor differentiation. Overall the presentation is well organized and data convincing.

This reviewer has a few comments as follows: 1. Major: It would be nice to show functional consequence of HNRNPH1 overexpression in human ESCC. For example, the authors may look at whether overexpression is associated with apoptosis, gene splicing, and differentiation using tissue microarray or TCGA data. They may also look at whether overexpression may be associated with poorer survival. Without such data, it is difficult to appreciate the significance of HNRNPH1 overexpression.

Response: We thank the reviewer for this helpful suggestion. In this study, we found that HNRNPH1 mRNA and protein was overexpressed in ESCC and associated with poor tumor differentiation. To further investigate the potential roles of HNRNPH1 in ESCC, we performed correlation analysis based on TCGA data. It was reported that HNRNPH1 modulates the alternative splicing of apoptotic mediator Bcl-x and A-raf[1,2]. In addition, the cell proliferation marker, MKI67 which encodes Ki-67 protein, was also included. As shown in our new Figure 5A and 5B, variant 2 of HNRNPH1 was positively correlated with the expression of MKI67 (Rho=0.3101, P=0.0035) and the prominent transcript of A-raf (Rho=0.2787, P=0.0090). Moreover, variant 2 was reversely correlated with the pro-apoptotic transcript of Bcl-X (Bcl-X(S), Rho=-0.2349, P=0.0285; Figure 5C).

Moreover, all of the cases were dichotomized into two groups, high level group and low level group, by the median RPKM values in tumor tissues. However, the mRNA levels of HNRNPH1 was not found to be associated with the overall survival of ESCC patients (Supplementary Figure S1). Therefore, the overexpression of variant 2 of HNRNPH1 was associated with cell growth and anti-apoptosis in ESCC. We had added one figure, one supplementary figure and description in the RESULTS and DISCUSSION sections.
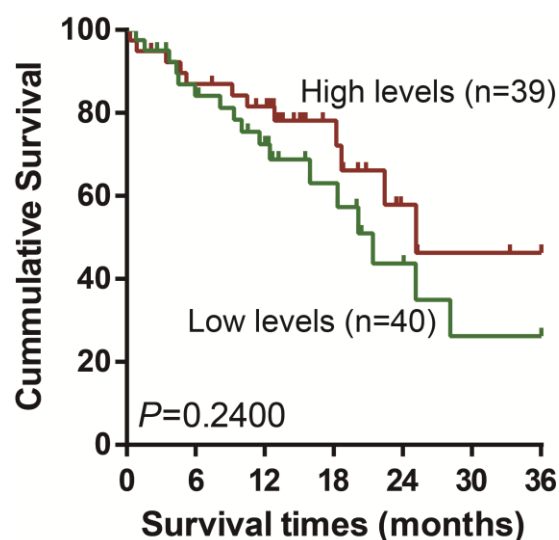
Supplementary Figure S1 Kaplan–Meier curves of ESCC patients with low and high levels of variant 2 transcript of HNRNPH1 (n = 79). All of the cases were dichotomized into two groups, high level group and low level group, by the median RPKM values in tumor tissues. Log-rank test was used to compare the survival curves (P = 0.2400).

1   Rauch J, O'Neill E, Mack B, Matthias C, Munz M, Kolch W, Gires O. Heterogeneous nuclear ribonucleoprotein H blocks MST2-mediated apoptosis in cancer cells by regulating A-Raf transcription. Cancer Res 2010; 70(4): 1679-1688 [PMID: 20145135 PMCID: PMC2880479 DOI: 10.1158/0008-5472.CAN-09-2740]

2   Garneau D, Revil T, Fisette JF, Chabot B. Heterogeneous nuclear ribonucleoprotein F/H proteins modulate the alternative splicing of the apoptotic mediator Bcl-x. J Biol Chem 2005; 280(24): 22641-22650 [PMID: 15837790   DOI: 10.1074/jbc.M501070200]

2. Figure 3 needs to be semi-quantitated by comparing the ratio of hNRNPH1/beta-actin in cancer versus normal. A bar graph with statistical analysis would strengthen this manuscript.

Response: We thank the reviewer for these critical comments. We had performed the densitometric analysis, and Figure 3 had been updated.

3. Table 1 pooled well and moderately differentiated ESCC as one group. It is better to split them into two groups and compare among well, moderately and poorly differentiated ESCC.

Response: We thank the reviewer for these comments. When we compared among well, moderately and poorly differentiated ESCC groups, the P value was 0.0652, and it was only marginal association. So we pooled well and moderately differentiated ESCC together, the P value reached to 0.0337, and it was more significant than the former. The revised Table 1 includes these two comparisons.

4. Table 1 is supposed to have 125 cases. Why are there only 42 with LN metastasis

and 36 without (in total 78 cases)?

Response: We thank the reviewer for highlighting this issue. Among the used 125 cases, only 78 cases had the information of LN metastasis. We had added a footnote in Table 1.

5. English needs to be edited.

Response: We thank the reviewer for highlighting this issue. We have carefully proofread the revised manuscript to minimize the typographical, grammatical, and bibliographical errors.

In addition, for the revision checklist, due to the Language Grade of our manuscript is A, it is not necessary to provide a language editing certificate based on the policies of World Journal of Gastroenterology. We had prepared the other

1 25957-Revised manuscript

    We had revised your manuscript according to the reviewers' comments and re-formatted the manuscript according to the Guidelines and Requirements for Manuscript Revision-Basic Study.

2 25957-Answering reviewers

    We had prepared the response letter.

3 25957-Copyright assignment

    We had prepared the scanned signed copyright assignment.

4 25957-Audio core tip

    We made an audio file describing the final core tip of the manuscript.

5 25957-Institutional review board statement

    This study was approval by the Institutional Review Board of the Cancer Hospital of Chinese Academy of Medical Sciences. The approved number is 12-130/664.

6 25957-Institutional animal care and use committee statement

    This study was not involved in animal experiments.

7 25957-Animal care and use statement

    This study was not involved in animal experiments.

8 25957-Biostatistics statement

    The statistical methods of this study were reviewed by Dr. Qingkun Song from Department of Cancer Research, Beijing Shijitan Hospital, Capital Medical University. He is the specialist in Epidemiology and Biostatistics.

9 25957-Conflict-of-interest statement

    We have no conflict of interest to declare.

10 25957-Data sharing statement

    Technical appendix, statistical code, and dataset available from the corresponding author at zhaoxh@cicams.ac.cn. Participants gave informed consent for data sharing. No additional data are available.

11 25957-Google Scholar

We searched our final title in Google Scholar, and we did not find any similar work.

12 25957-CrossCheck

We did not use the CrossCheck program this time. We can declare that there is no plagiarism and fake data in our manuscript. If the editor thought that it is necessary, we can do it.

13 25957-Language certificate

To minimize the typographical, grammatical, and bibliographical errors in this manuscript, we asked for a full professor who are in the Canada to proofread it. Some mistakes had been fixed.

Thank you and all the reviewers for the kind advices.

Sincerely yours,

Xiaohang Zhao
Professor of National Cancer Center/Cancer Institute of CAMS