

December 20, 2018

Subject: Revision and resubmission of manuscript NO 44514

Dear Dr. Lian-Sheng Ma

Thank you for your letter and this opportunity to revise our paper “Overexpression of HSP27 and HSP70 is associated with decreased survival among patients with esophageal adenocarcinoma”. The comments and suggestions offered by the reviewers were immensely helpful, and we have revised our manuscript accordingly. Our responses are given in a point-by-point manner below.

We hope the revised version is now better suited for publication but are happy to consider further revisions. We thank you for your continued interest in our research and look forward to hearing from you in due course.

Sincerely,

Reviewer #1 comment:

1. The authors had better describe about the potential efficacy of the HSP27/70 for clinical decision making for EAC treatment. Therefore, the authors should add and show subgroup analysis about clinical stages and kinds of treatment.

Response: Thank you for this very helpful comment. We reviewed our data accordingly, and found in subgroup analysis that stage II cancer patients with high HSP27/70 expressions had significantly poorer prognosis compared to patients with low expressions. This could potentially make them a beneficiary target group

for HSP inhibitors, although the hypothesis needs further clinical testing. We also found that patients unsuited for surgical treatment were more likely to have high HSP expression, implying more aggressive disease. These results have been added to the manuscript.

Reviewer #2 comments:

1. IHC pictures showing excellent IHC quality should be included

Response: Unfortunately, we are not able to acquire such pictures. We contacted the pathologist who was not able to locate them. We sincerely apologize for this, as we too had wished to publish them.

2. The reviewer posed question about TMA tissue core size, thickness of microscope slides and who observed the IHC stainings.

Response: We discussed these issues with our pathologist, who confirmed that the thickness of all slides was 5µm. We will take into consideration the TMA tissue core size in our future studies. We clarified the question about the observers in the chapter "Evaluation of HSP27 and HSP70 expressions", and all unclear or borderline cases were evaluated by an experienced GI pathologist. All cases were primarily evaluated by two medical residents (not pathologists) as part of their theses.

3. Why 1.5 was used for the cutoff?

Response: Clarification has been added to the manuscript. HSP expression was quantitative, and therefore we chose the median of the range to divide cases into "low" and "high"

4. How to define the 85% success in cancer sample and 50% in BE?

Response: We are extremely grateful for this comment, as it made evident a significant misunderstanding on our part. Our first author was not involved in the original patient gathering and IHC analysis, and therefore she was unaware that “unsuccessful” in our data meant that there was not enough tissue left for HSP analysis. The original study group that collected the then available samples did unrelated IHC, using all of the biopsy material in some of the cases, hence rendering them unavailable for HSP analysis. All samples we had available were “successful”, either negative or on a scale of 1 to 3. The BE samples were all from small biopsies so many of them were unavailable for HSP analysis, and since BE was not the study objective, we chose not to acquire more samples. The revised number of BE samples is 15.

We sincerely apologize for this oversight on our part, and have revised the manuscript, Table 1 and Figure 1. We changed “success” to “available”, excluded the cases from our demographics and revised both abstract and manuscript so that these unavailable cases are not included.

Reviewer #3 comments:

1. There was no statistical difference in staining frequency of HSP27 between cancer cases and Barrett’s esophagus and control cases but there was correlation between staining frequency of HSP27 and survival?

Response: Thank you for an interesting question. Although there was no significant difference in staining frequency, there was a trend towards higher HSP27/70 expression in BE with high grade dysplasia. One possible explanation for the lack of significant differences is the small number of BE (15) and control (5) samples.

2. According to tables 2 and 3, tumor stage and HSP expression seems to be correlated but you confirmed that tumor stage did not correlate with HSP. This result must be shown.

Response: Thank you for this comment, we have revised our manuscript to show this more clearly. Although the correlation between stage and HSP staining was not statistically significant, we found in subgroup analysis that high HSP27/70 expression in Stage II cancer correlated with a significantly poorer survival. This result has also been added.