December 20, 2014

Dear Editor,

Please find enclosed the revised manuscript in Word format (file name: 15028-review.doc).

Title: MMS19 expression is associated with metastasis and chemoradiotherapy response in esophageal cancer

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 15028

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers

ANSWER FOR REVIEW AND COMMENTS (02978590)

Thank you for your kind review and we are really grateful to your comments that "the study is with some potential to open up new lines of research".

Comment 1: Was normal esophageal control biopsies obtained from cancer patients' esophagus (which is partly implied) or from other patients?

Answer: The normal esophageal control biopsies obtained from cancer patients' esophagus. To be more specific, we modified the related sentences as "52 samples of

normal esophageal squamous epithelia (NESE) were biopsied from those of 103 ESCC patients at least 5 cm from the primary lesion."

Comment 2: Has the employed method of detecting and quantifying MMS19 been validated with some other method? Please give reference. Moreover the composite score used for quantification seems to have been constructed by the authors. Has it been validated? If not, please discuss this in a clearer way.

Answer: Using Northern blot, the study performed by Seroz et al [1] demonstrated that MMS19 gene was expressed at a rather low level in all organs and tissues such as brain, kidney, heart, liver and lung excepted testis. Another study revealed that MMS19 mRNA was expressed in moderate to high steady-state levels in human adult and fetal tissues with Northern blot [2]. The result of our study that expression of MMS19 is relatively low in normal tissues is in accord with these studies. Wu et al [3] have investigated the expression status of MMS19 in cancer cell lines as HL60, Hela, K562, MOLT-4, Raji, SW480, A549 and G361 by Northern blot. However, there are rare studies to compare the expression level of MMS19 between normal and cancer tissues in any means, which intrigued us most. The method of immunohistochemical staining for detecting and quantifying protein in cancer has been widely used in cancer research and clinical practice, as was applied in studies of [4-8]. Thus, we did not validate MMS19 expression level in ESCC by other method. Besides immunohistochemical staining, Western-blot was often used to evaluate proteins expression, however, this method was always applied to evaluate the protein in whole cells, which may not well reflect the clinical significance of subcellular protein distribution as we expected. The composite score for quantification of MMS19 expression is not created by us, and we just quantify MMS19 expression with the composite score according to the method described by other researchers (see reference [26]). In the revised manuscript, we add a sentence "The quantification of MMS19 expression was performed according to a previous study [26]" in the section of "***Immunohistochemical staining***".

Comment 3: In Table 1, quantification of MMS19 expression using the 12-grade composite scale is only showed categorized as high and low expression. Please show actual score for

each category.

Answer: we modified the expression "Then, a composite score ranging from 0 to 12 was obtained. Based on the final score, each case was divided into a high expression group (≥ 6) or a low expression group (< 6)" as "Then, a composite score scaled as 0, 1, 2, 3, 4, 6, 8, 9, and 12 was obtained. Based on the final score, each case was allocated into a high expression group when the score was ≥ 6 (6, 8, 9 and 12) or a low expression group when the score < 6 (0, 1, 2, 3 and 4)". In each table, we add footnotes to explain score range of the "high expression" and "low expression". Further, we supplement the mean score of each group " The mean (±SE) score of cytoplasmic MMS19 expression in the high expression group and low expression group was 7.78 (±0.274) and 2.79 (±0.214), respectively. Whereas, the mean score of nuclear MMS19 expression in the high expression group and low expression group was 6.86 (±0.315) and 2.68 (±0.141), respectively." in RESULTS part.

Comment 4: In the results section as well as in the tables 1 and 2 it is not at all clear whether the tumor tissue used is from endoscopic biopsies or from operative specimens. Please clarify this.

Answer: Now we modified the titles of results in the Results section and titles of table 1 and 2, which clearly stated that the results were obtained from biopsied or resected samples.

Comment 5: Why was the cut-off level for age chosen to be 55 years?

Answer: Using 55 years old as the cut-off was based on a previous study [9]. Age was always as a baseline clinicopathological factor measuring the homogeneity of data between different groups. Various cut-offs for age as 40[10], 50[11], 56[8], 60[12], 61[13], 62[14], 63[15], 65[16], 70[17] and 69[18] years could be found.

Comment 6: Language is substandard. Please let native English speaker revise manuscript.

Answer: The language have been polished by native English speakers and a certification is received.

ANSWER FOR REVIEW AND COMMENTS (02446765)

Thank you for your kind review and comments and we really appreciate your efforts.

Comment 1: classification of response to chemotherapy has not been clearly defined and only 29 and 20 patients were enrolled in good response group and poor response group. Please describe more precisely about the definition.

Answer: The vague definition of response to chemotherapy may cause by arrangement of text structure. In MATERIALS AND METHODS part, we defined the histopathological response of surgical specimens as four types of chemoradiotherapy response: "The histopathological response to CRT was evaluated by two experienced pathologist according to previously published criteria [24, 25]. The percentage of residual viable tumor cells was estimated, and each patient was subsequently allocated to one of the following 4 groups: complete response group, no residual tumor cells; major response group, <10% residual tumor cells; partial response group, 10-50% of residual tumor cells; minor response group, >50% of residual tumor cells." And we further defined the chemoradiotherapy response as good response group and poor response group in RESULTS part as "For the statistical analysis, the patients were divided into two groups according to CRT response: a good response group, consisting of patients with a complete response and major response; and a poor response group, including patients with a partial response and a minor response."

To avoid confusion, we merged the two parts in MATERIALS AND METHODS section as "The histopathological response to CRT was evaluated by two experienced pathologist according to previously published criteria [24, 25]. The percentage of residual viable tumor cells was estimated, and each patient was subsequently allocated to one of the following 4 groups: complete response group, no residual tumor cells; major response group, <10% residual tumor cells; partial response group, 10-50% of residual tumor cells; minor response group, >50% of residual tumor cells. For the statistical analysis, the patients were divided into two groups according to CRT response: a good response group, consisting of patients with a complete response and major response; and a poor response

group, including patients with a partial response and a minor response." In order to keep the consistency, we add "Thus the good response group and poor response group included 29 and 20 cases, respectively." in RESULTS part.

Comment 2: Only immunohistochemical staining was performed to evaluate the expression of MMS19. Inter-observer variation is quite common with this method. How did the authors try to overcome this limitation?

Answer: Indeed, inter-observer variation is quite common with immunostaining. the method of immunohistochemical staining for detecting and quantifying protein in cancer has been widely used in cancer research and clinical practice, as was applied in studies of [4-8]. In this study, in order to overcome this limitation, immunostaining was evaluated by two experienced pathologists. If different scores for a same sample were made by the two pathologists, this sample would be revaluated again by them. If the score was still not consistent, the two pathologists would discuss and decided a final score. With this procedure, the staining score will reflect the immunostaining as real as possible. We supplement this detail of score evaluation in ***Immunohistochemical staining*** part.

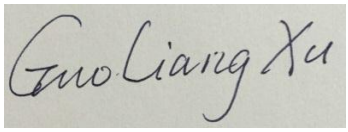
Comment 3: Please describe full terms of each abbreviations below tables.

Answer: Full term of each abbreviation below tables was described as your comment.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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