Sep 20, 2016

Ze-Mao Gong, Science Editor,

Damian Garcia-Olmo, Stephen C Strom, Andrzej S Tarnawski, Editors-in-Chief,

World Journal of Gastroenterology

Manuscript No.: 29596

Dear Editors,

Please find attached our manuscript entitled "Prognostic implications of *FGFR1* and *MYC* status in esophageal squamous cell carcinoma" by Dohee Kwon, *et al.*, which we are submitting for consideration for publication as a Retrospective Study in *World Journal of Gastroenterology*.

This manuscript had been previously submitted to *World Journal of Gastroenterology,* in your letter of Sep 6, 2016, you indicated that the reviewers felt the manuscript has merit and advised us to revise our manuscript and that the Editors would reconsider a revised manuscript for publication, pending on revision required. We wish to express our gratitude to you and the reviewers for the careful review of our manuscript. We addressed the concerns of the reviewers and made a few corrections and clarification in the manuscript after going over the reviewer's comments.

Please see the enclosed our answering reviewers file for responses to Reviewers' Comments. We also carefully checked and uploaded all files and documents required for manuscript revision. Please also see the PowerPoint (PPT) file for

figures, uploaded separately. In addition, we are sorry that grant application (approval) forms are seen in Korean, because English version of grant forms are not available.

We hope that the incorporated changes and additions allow the manuscript to be suitable for publication in *World Journal of Gastroenterology*. We greatly appreciate in advance your consideration of our work and look forward to hearing from you.

Best wishes,

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Responses to reviewers' comments

Responses to comments by reviewer 02922262

It is a very interesting article presenting novel data on role of FGFR1 and MYC status in ESCC. All parts of the manuscript were composed correctly and they contain suitable information. Tables and figures were constructed appropriately. Statistical

analysis of data was performed correctly with using the appropriate tests. All references are actual and relevant to the text of article. In my opinion manuscript can be accepted for publication in the World Journal of Gastroenterology.

; We'd like to appreciate you for the time and effort on reviewing our manuscript.

Responses to comments by reviewer 01329728

The manuscript by Dr. Dohee Kwon et al., reports that FGFR1 amplification and MYC expression affects ESCC prognosis and implicates the target of the therapy. The manuscript is well and concisely written and discussing point is almost clear. The manuscript should, however, address the following issues:

1) Authors described that patients were divided with two groups, (MYC expression vs No expression) and MYC expression group is associated with good prognosis of ESCC patients. This is the results of grade 0 vs 1-3 in MYC IHC. Is this division good? How about 01 vs 23 or 0-2 vs 3?

; We'd like to appreciate you for all the valuable comments. We had performed survival analysis after dichotomizing the patients according to variable cutoffs for MYC IHC grade including 0-2 vs 3, 01 vs 23, and 0 vs 1-3. By doing this, we tried to determine MYC IHC cutoff value with most consistently lowest P values in terms of prognostic significance. In brief, patients with MYC IHC grade 3 were small (n=11/180 [6.1%]) and thus there was no statistically significant difference in the survival compared to those with MYC IHC 0-2. When dichotomizing patients into MYC IHC 0-1 vs. 2-3 groups, those with MYC IHC 2-3 showed better disease-free survival (DFS) and overall survival (OS) (P=0.02 and 0.002, respectively). However, there was a crossing at the Kaplan-Meier survival curves for DFS. In addition, OS was not significantly different between MYC IHC 0-1 and 2-3 groups in patients who did not receive adjuvant therapy. In contrast, patients with MYC IHC 1-3 showed

significantly longer DFS and OS compared to those with MYC IHC 0 in whole cohort (P=0.036, P=0.017, respectively) and in the cohort with no adjuvant therapy (P=0.032 and P=0.031, respectively). Thus, we comparatively analyzed the survival of patients after dichotomizing patients into MYC IHC 0 vs. 1-3 for MYC expression.

2) We understand that MYC functions as oncogene. However, the authors described MYC expression affects better prognosis of ESCC patients. They should mention about this discrepancy more in Discussion.

; Thank you for the important comments. As you point out, increased copy number or overexpression of MYC have been reported as poor prognostic factors in variable cancer types. Although increased MYC copy number measured by array-CGH or qPCR were poor prognostic indicators in ESCC (Cancer Sci 2012;103:1558), a few reports are available for the prognostic significance of MYC expression in ESCC. In this study, MYC expression but not amplification (measured by FISH) was associated with prolonged survival of patients with ESCC in univariate analysis. However, in this study, MYC expression was more common in ESCC patients of younger age and in the early TNM stage, and it was not an independent prognostic factor in multivariate Cox analysis (Table 4). Thus, we think that MYC might have an oncogenic role in ESCC but have a little, if any, prognostic implication in patients with ESCC. More studies using a large cohort of patients would be needed to validate the prognostic significance of MYC in ESCC. We added a sentence that MYC expression lost its prognostic significance in multivariate Cox analysis in the Result section, and discussed the above discrepancy in the revised manuscript in addition to Discussion, as you recommended.

3) Why there are differences of prognosis in only no adjuvant therapy group?

; We think that you pointed out a relevant issue. Please let us answer to this

comment in a speculative manner as previously discussed in the manuscript, because, unfortunately, we don't have any experimental data to explain those observations. First, in this study, patients with adjuvant chemo- and/or radiotherapy tended to be in the advanced stage compared with those with no adjuvant therapy. Thus, FGFR1 might play variable biological roles during the progression of cancer and thereby have different prognostic significance depending on the stage and subsequent adjuvant therapy status of patients. Second, it could be possible that ESCC with FGFR1 amplification represents a biologically less aggressive group among ESCCs having variable genetic alterations. This could result in the prolonged survival of patients receiving no adjuvant therapy. Last, FGFR1 could affect the efficacy of chemo- or radiotherapy in patients with ESCC, and thus be differently associated with the prognosis in those receiving adjuvant therapy. However, this study had some limitations in that it was a retrospective study, as such, another study using large prospective cohorts is required to validate the prognostic role of FGFR1 amplification in ESCC according to adjuvant therapy status. We added the above description in the Discussion of revised manuscript.

Responses to comments by reviewer 00052396

This manuscript by Kwon et al is a straightforward clinical study investigating the prognostic implications of FGFR1 and MYC status in ESCC. The findings parallel what has been reported in multiple other malignancies and ESCC. No particularly novel information is provided in the manuscript. In general, the manuscript is descriptive in nature and lacks innovation. There are three technical issues:

; Thank you so much for your comments. Please see the below for responses to each comment. We'd like to say that we included the limitation of this study due to the technical issues you raised in the Discussion of revised manuscript.

(1) It is not clear exactly how these patients are followed up.

; ESCC patients in our institute have been treated as follows; 1) operation only, 2) operation and adjuvant radiotherapy, 3) operation and adjuvant chemotherapy, 4) operation and adjuvant concurrent chemoradiotherapy (CCRT), 5) neoadjuvant CCRT and operation. We excluded patients who had received neoadjuvant therapy. Furthermore, we grouped patients into operation only cohort (i.e., "no adjuvant therapy cohort") and adjuvant therapy cohort (i.e., "adjuvant chemo- and/or radiotherapy cohort"). As described in the manuscript, the mean follow-up period was 43.22 months (ranges 0.6-169.4 months) in whole cohort; 47.55 months (ranges 0.6-169.4 months) and 34.12 months (ranges 4.2-131.7 months) in no adjuvant therapy cohort and adjuvant chemo- and/or radiotherapy cohort, respectively. However, we agree with you that this study has limitation in that it is a retrospective study.

(2) Some of these tissue samples date back to the year of 2000. Validation of tissue quality is essential to ensure that a negative staining is truly negative. 2 mm core was used to represent a case instead of tissue sections. It may create false outcome due to tumor heterogeneity.

; We agree with you that FFPE blocks in archives could have quality issues and 2mm-core TMA samples don't represent the whole tumor. First, the quality of old FFPE tissue was ensured by checking the FISH signals for FGFR1 and MYC in overall area of each core and by evaluating the MYC staining in basal cells of adjacent epithelium. The heterogeneity issue might be reflected by results of FGFR1 and MYC status between primary and metastatic tumor in lymph node (Table 3). Notably, FGFR1 amplification status was not statistically different between primary and nodal metastatic tumors, but MYC amplification was inconsistent between

primary and nodal metastatic tumors.

(3) Sample size needs to be justified in particular when small groups are compared (e.g., Figure 2K).

; In this study, some patient groups were small in size and had censored data. Therefore, we might not avoid type-two error (false negative) in the Kaplan-Meier analysis, because it does not have enough power to rule out a real difference. However, in the Cox multivariable analysis, log minus log graph comparing combined positivity and the others showed no crossed points of the lines especially in adjuvant chemo- and/or radiotherapy cohort. Thus, we think that combined positivity has steady effect on the mortality, even though it does not have significant P value.

Responses to comments by reviewer 02446446

The manuscript of "Prognostic implications of FGFR1 and MYC status in esophageal squamous cell carcinoma" is reasonably interesting and I definitely recommend publication. I, as a pathologist actually enjoyed reading your manuscript. I believe that, the results of your study which conclude that FGFR1 amplification and MYC expression has prognostic implications in resected Esophageal Squamous cell Carcinoma (ESCCs) with respect to adjuvant therapy. Your study clearly demonstrates that: 1. MYC expression was associated with prolonged disease-free survival and overall survival (OS) but was not an independent prognostic factor. 2. FGFR1 amplification was an independent predictor for prolonged OS in all patients and in those who did not receive adjuvant therapy. 3. Combined FGFR1 amplification and MYC expression predicted better OS in patients who did not receive adjuvant therapy but not in those who did receive adjuvant therapy. The

manuscript is very well written and I have no issues with this paper.

; We'd like to greatly appreciate you for the careful review of our manuscript and encouraging comments.