Format for ANSWERING REVIEWERS

August 31, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12053-edited_review.doc).

Title: Prognostic value of *KRAS* and *BRAF* mutations in curatively resected colorectal cancer.

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Name of Journal: *World Journal of Gastroenterology*

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

1. Format has been updated

(1) We have made use of a language editing service provided by a professional English language editing company (Enago), and the editorial certificate was attached.
(2) Requirement for decomposable figures: We changed the figure format to Windows Enhanced Metafile in the Word file. We additionally attached the ppt. file. Please let me know if there are any problems.
(3) Changes made in the revised manuscript have been highlighted.

2. Revision has been made according to the suggestions of the reviewer

Comments by reviewer No.69946

(1) Indeed MSI-H patients are generally considered to be in good prognosis, but the results of this study does not correspond to these previous reports, suggesting that they may leads by the difference of background in registration CRC cases?

Response

The present study included consecutive patients with stage I to III CRC who underwent curative resection at our institute; therefore, it is quite unlikely that this result was attributed to selection bias. As shown in Table 3, MSI-H tumors showed non-significant trends toward better disease-free survival (DFS) (HR = 0.64) and overall survival (OS) (HR = 0.81); however, the wide confidence interval makes it to definitively conclude the prognostic impact of MSI status. Therefore, additional larger studies are needed to clarify the prognostic impact of MSI status. We described this limitation in discussion (page 14; lines 17-21).

(2) Is there any reports concerning to the gender difference in *KRAS*/*BRAF* mutation incidence in the previous CRC research? The authors should discuss them in the manuscripts.

Response

As mentioned in discussion (page 12; lines 8-18), there are no consistent results regarding the gender difference in *KRAS* mutation. As for *BRAF* mutation, the systematic review including 9,885 patients showed that *BRAF*-mutated colorectal cancer is associated with proximal tumor location, T4 tumors, poor differentiation, female sex and so on (Clancy C, et al. Colorectal Dis 15:e711-8, 2013). We added this article to references and discussed in the manuscript (page 13; lines 20-24).

(3) How about the anticancer drug sensitivity, especially EGFR inhibitor, in your institute? Please add the consideration with preliminary data.

Response

The present study targeted curatively resected colorectal cancer; therefore, we could not evaluate the anticancer drug sensitivity in this cohort.

Comments by reviewer No.48795

(1) As the authors mentioned, in contrast to previous reports, their analysis did not show that patients with MSI-H tumors exhibited better survival than those with MSS/MSI-L tumors. Dose the difference of the previous reports depend on the racial and/or environmental differences between Western and Asian populations, or the small number of patients with MSI-H tumors in this study? Additional larger studies may needed to clarify the modifying effect on the relation between *BRAF* mutations and survival outcome according to MSI status. These minor comments however shouldn't detract from well written report.

Response

As shown in Table 3, MSI-H tumors showed non-significant trends toward better DFS (HR = 0.64) and OS (HR = 0.81); however, the wide confidence interval makes it to definitively conclude the prognostic impact of MSI status. Therefore, additional larger studies are needed to clarify the prognostic impact of MSI status. We described this limitation in discussion (page 14; lines 17-21).

Comments by reviewer No.928913

(1) The major point that authors have to elucidate is why they analyze the prognostic roles of both *KRAS* and *BRAF* mutation status after adjustment for microsatellite instability (MSI) status. KRAS mutation is categorized to chromosome instability (CIN) pathway of CRC tumorigenesis, while *BRAF* mutation is categorized to MSI pathway of CRC tumorigenesis.

Response

KRAS mutation can occur in MSI-H tumors, and *BRAF*-mutated tumors sometimes display CIN (reference: Asaka, et al. Carcinogenesis 30: 494-499). Therefore, it is difficult to simplify as above commented.

(2) In the Materials and Methods section, there were 813 tumor samples were used for subsequent analysis. However, in Table 1, there was one missing tumor tissue in *KRAS* mutation analysis and two missing tumor tissues in *BRAF* mutation analysis. The reason for the inconsistent data in the text and Table 1 should be proposed. Also, the incidence of MSI-H was relatively low than previous publications.

Response

As mentioned in the results section (page 10; lines 4-5), sufficient samples were not available for determining mutational status (1 case for *KRAS* and 2 for *BRAF*).

(3) In Figure 1, Kaplan–Meier curves for DFS and OS according to *KRAS* or *BRAF* status. Presence of *BRAF* mutations was not significantly associated with poorer DFS and OS in the entire cohort (Figure 1; Table 2). On the contrary, *BRAF* mutation status was

prominently associated with DFS and OS in Cox proportional models (Table 3). Consequently, the contradictory role of *BRAF* mutations in DFS and OS must be elucidated and discussed in the Discussion section.

Response

In univariate analysis, *BRAF* mutation was not associated with DFS and OS, but trends toward inferior DFS and OS were observed in the Kaplan–Meier curves (Figure 1). *BRAF* mutations were frequently observed in female and MSI-H tumors which showed trends toward better DFS and OS in our cohort. Adjustments for these relevant factors mainly modified the prognostic impact of *BRAF* mutations in Cox proportional models.

(4) MSI-H tumors were only 8.2% in the current study, of which was considerably lower than the approximately average 10-15% than previous study. If it is possible from the PCR analysis on ISH method they used. In addition, in table 2, authors classified tumor location to proximal, distal and rectum. However, according to recent gene signature/pathway differences, now it is the trend to category CRC tumors to left vs. right colon tumors of patients.

Response

As mentioned in the discussion section (page 13; lines 16-26, page 14; lines 1-3), the frequency of MSI-H in our cohort was lower than that in Western populations (11-17%) and comparable with that in Asian populations (6-12%). This discrepancy in MSI-H status between Western and Asian populations may be attributed to the different distribution of patients' characteristics such as gender, tumor location, histological grade, or racial and/or environmental differences. As for tumor location, the results have not changed when categorized to left vs. right CRC tumors.

(5) MSI-H patients were demonstrated to have no advantage of receiving adjuvant chemotherapy in colon cancer patients. If the similar findings are also observed in the current study?

Response

Adjuvant chemotherapy was not performed for patients with stage I disease. In contrast to previous reports, adjuvant chemotherapy tended to improve DFS (univariate HR = 0.59; P = 0.34) and OS (univariate HR = 0.43; P = 0.16) in MSI-H patients with stage II and III disease. These findings may be attributed to patient selection bias.

(6) The effect of *KRAS* mutations on DFS and OS was limited to patients with MSS/MSI-L tumors. If the addition of adjuvant chemotherapy would play a role among these patients?

Response

Adjuvant chemotherapy provided significant improvement in DFS (univariate HR = 0.68 and 0.55; P = 0.049 and 0.02, respectively) and OS (univariate HR = 0.44 and 0.40; P = 0.006 and < 0.001, respectively) in patients with stage II and III disease.

(7) Despite the BRAF mutation data from Taiwan and Japan were discussed, authors just cited the studies of *KRAS* mutations on DFS and OS of Caucasian CRC patients, but no relevant study from Asian countries. The differences between Caucasian CRC and Asian CRC patients are suggested to be mentioned in the Discussion section. Recently, an article published from Asian country, similar to the PETACC-3 trial, their results showed that there is no significantly different between *KRAS* wild-type and *KRAS* mutation UICC stage I-III CRC patients, of which is suggested to cite it (BMC Cancer. 2013 Dec 13;13(1):599). Thus, the discrepancy in *KRAS* mutations status between Western and Asian populations may be crucial.

Response

According to the reviewer's comment, we described two relevant studies from Asian countries in the discussion section (page 12; lines 20-26). The article which the reviewer presented deals with only metastatic disease and seems to have no application in this case.

(8) Please uniform the word type regarding gene name as an Italic letter.

Response

According to the reviewer's comment, we uniformed the gene name as an Italic letter.

Comments by reviewer No.1714224

(1) The main limit of the study is the lack of any information regarding which kind of *KRAS* mutations have been assessed. It is well known that a large amount of literature has been published regarding the role of *KRAS* mutations, especially in codons 61 and 146, but also in codons 12, 13 and 117. Different mutations have been related to a different outcome of disease (see for example, only demonstrative and not exhaustive, a recent paper by Imamura et al. "Analyses of clinicopathological, molecular, and prognostic associations of *KRAS* codon 61 and codon 146 mutations in colorectal cancer: cohort study and literature review" in Mol Cancer 31;13:135, 2014.). Authors should furnish data regarding the codons assessed for *KRAS* mutations, survival and disease free data have to be evaluated taking into account the codons analyzed.

Response

We previously found no difference in survival outcomes between specific *KRAS* mutations including exon 2, 3 and 4 (Ogura T, et al. Oncology Reports 32: 50-56, 2014). Therefore, we did not take into account the specific codons for *KRAS* mutations. In the revised version, we added the context mentioned above in the discussion section (page 13; lines 2-5).

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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