ANSWERING REVIEWERS

Novemver 14, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14557-Review.doc).

Title: Mucinous phenotype and CD10 expression of primary adenocarcinoma of the small intestine

Author: Reiko Kumagai, Kenichi Kohashi, Shunsuke Takahashi, Hidetaka Yamamoto, Minako Hirahashi, Kenichi Taguchi, Kenichi Nishiyama, Yoshinao Oda

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14557

The manuscript has been improved according to the suggestions of editor and the revision policies for original article:

1 Format has been updated

(1) We made the title shorten.

"Mucinous phenotype and CD10 expression of primary adenocarcinoma of the small intestine: Possible association with biological behavior, genetic alteration and microsatellite instability status"

→"Mucinous phenotype and CD10 expression of primary adenocarcinoma of the small intestine"

(2) We changed the running title as below

"SIAs and phenotypic expression"

→"Phenotypic expression of Small intestinal adenocarcinoma"

(2) We added core tips. (page 4 line 18 to page 5 line 3)

(3) We changed the expression of statistical data as mean±SD. (page 4 line 1, page 4 line 5, page 13 line 4, page 13 line 8, page 13 line 18, Table 3)

(4) We eliminate p-value more than 0.05. (page 13 line 7, page 13 line 9, page 13 line 20)

2 Revision has been made as below, according to the suggestions of the reviewer 00033010

(1) Some abbreviations are not fully explained.

Replay: We found lack of explanation, and explained all abbreviations.

"**AIM**: To clarify the correlation with the phenotypic expression…."

→"**AIM**: To clarify the correlation with phenotypic expression, clinicopathological features, genetic alteration and microsatellite-instability status in small intestinal adenocarcinoma (SIA)."(page 3, line 4)

(2) Authors should clarify why they considered as negative controls for mismatch repair protein expression, the normal tissue close to cancer of resected specimens, instead of small bowel tissue from healthy population.

Replay: The way as your suggestion may be better to evaluate immunohistochemical stain of mismatch-repair proteins (MLH1, MSH2). In some papers, authors used the adjacent normal mucosa as internal control (Fu T et al. Clin Cancer Res. 2012, Yasugi A et al. Oncol Rep. 2008, Erdamar S et al. World J Gastroenterol 2007). We choose the specimen which had enough amount of the normal mucosa as well as adenocarcinoma area. We confirmed that the nuclei in normal mucosa showed positive staining, then evaluate the expression of mismatch-repair proteins (MLH1, MSH2).

(3) Authors should report clearly the correlation between mucins/CD10 expression and TNM stage. Although they reported the main differences in Table 3, a discussion in the text is fundamental, and should be supported by a statistical comparison. This aspect is lacking in the text.

Replay: We made a statistical comparison according to your comment, and added the result in the manuscript and Table 3.

→"The invasion depth was significantly deeper in the CD10(−) group than in the CD10(+) group (T1 vs T2-T4$p<0.05$)." (page 13 line 18 to line 20)

(4) Figure 1A: an arrow highlighting CD10+ cells may be useful.

Repay: We added arrow according to your comment.

(5) The intelligibility of CD10 staining in figures 1 and 2 is poor. Arrows denoting positive and negative staining are necessary, a higher magnification could be useful.

Repay: We took the micrographs again, and added arrows according to your comment.

3 Revision has been made as below, according to the suggestions of the reviewer 00225294

(1) Can the authors perform an additional linkage analysis of the clinical and personal parameters (age, sex, mutational analysis, etc.)

Replay: We made an additional linkage analysis according to your opinion and added the result in the manuscript and Table 5.

"… and no MMS tumors (Table 4). The MSI-H SIAs were composed of 2 well differentiated adenocarcinomas and 2 mucinous adenocarcinomas (Fig. 3)."

→"… and no MMS tumors (Table 4). The data of MSI status and clinicopathological features is shown in Table 5. The MSI-H SIAs were composed of 2 well differentiated adenocarcinomas and 2 mucinous adenocarcinomas (Fig. 3). The tumor size was significantly larger in MSI-H SIAs than in MSI-L and MSS SIAs (mean 7.7±1.1 cm vs 5.1±2.0 cm, $p<0.05$)."(page14 line 14, page 14 line 16 to line 18)

"…within codon 13. No BRAF V600E mutation was found in any cases."

→"…within codon 13. The data of KRAS mutation and clinicopathological features is shown in Table 5. No BRAF V600E mutation was found in any cases." (page 15 line 2 to line 3)

(2) Have the authors the possibility to incorporate other Ras alterations (Kras vs NRas) and p53. This is important in view of the literature regarding the biological outcomes of these adenos.

Replay: We agree with your opinion that it is better to add the molecular analysis of

these genes. We had already made mutational analysis of *p53* gene, however, it was not unclear the correlation among mucinous expression, CD10 expression and *p53* mutation. In addition, we don't have any gene probe to analysis *Nras* mutations. We beg your pardon that we are not able to perform the molecular analysis or clarify the incorporation of *p53* gene mutation, according to your suggestion.

4 We re-submitted this report to the professional linguistic revision, KN international. The certification was attached. (PDF: certificate of proofreading).

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

Sincerely yours,

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