August 30, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (12238-review.doc).

Title: ARID1A expression in gastric adenocarcinoma: clinicopathological significance and correlation with DNA mismatch repair status

Author: Ryo Inada, Shigeki Sekine, Hirokazu Taniguchi, Hitoshi Tsuda, Hitoshi Katai, Toshiyoshi Fujiwara, Ryoji Kushima

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The manuscript has been improved according to the suggestions of reviewers. Detailed answers to the reviewers' comments are provided below.

1. Format has been updated

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

3. References and typesetting were corrected

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

Sincerely yours,

Shigeki Sekine, MD, PhD
Molecular Pathology Division
National Cancer Center Research Institute, Tokyo, Japan
5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan
Telephone: +81-3-3542-2511 (ext.4210), Fax: +81-3-3248-2463
E-mail: ssekine@ncc.go.jp

Reviewer No. 02954962

This reviewer did not provide any specific comments.

Reviewer No. 02965551

(1) Stratify the age according to sex and then reanalyze the data. This might change the conclusions.

According to the reviewer's suggestion, we analyzed the correlations between the age and the statuses of MMR deficiency and ARID1A expression among male and female patients separately. As indicated below, the MMR deficiency was significantly associated with an older age among female cases. Among male patients, there was a similar tendency but the difference did not reach statistical significance. Regarding ARID1A expression, no significant differences were observed in either group.

In the univariate analysis, we had already shown that MMR deficiency was significantly associated with an older age and a female sex (Table 2). Therefore, we do not feel that this additional analysis altered the conclusions of our study significantly.

	Total	MMR		*P*-value	ARID1A		*P*-value
		Deficient	Intact		Loss/Weak	Retained	
Male							
Age, Mean±SD	61.4±11.0	66.2±8.0	61.1±11.1	0.078	64.0±10.9	61.4±11.1	0.837
Female							
Age, Mean±SD	60.4±13.1	70.5±9.8	59.0±12.9	<0.001	63.6±11.0	59.4±13.6	0.077

(2) Authors need to discuss their findings of very low proportion with MMR deficiencies

According to previous studies from Asian countries, the prevalence of MMR deficiencies is relatively low (less than 10%); thus, our result is consistent with those of previous studies. We have added a description concerning geographical variations in the prevalence of MMR deficiency to the revised manuscript (page 16, line 13).

Reviewer No. 00503536

(1) Although multivariate analysis in Cox's proportional hazard model show weak association between abnormal ARID1A expression and a poor

prognosis, abnormal ARID1A expression does not affect disease specific survival as shown in Fig.3. The interpretation of the data are confusing, and the authors should discuss more on that point.

We think that this issue is more likely to be related to the statistical methods in general rather than an issue specific to the present study. The Cox regression hazard model was used to control the effects of confounding variables; thus, the present findings suggest that abnormal ARID1A expression was shown to be associated with a poor prognosis only by adjusting for the effects of confounding factors.

(2)There are four different patterns of abnormal ARID1A expression as shown in Fig. 2, its association with lymphatic invasion, lymph node metastasis, prognosis or MMR deficiency is not demonstrated.

It has been shown that both a loss and a reduction in ARID1A expression suggest the presence of mutations in *ARID1A* (ref. 23). Based on this finding, we regarded these findings to be indicators of ARID1A inactivation. Another issue is that the number of cases with specific abnormality types was too small for their clinicopathological significance to be analyzed individually. For these reasons, we analyzed the cases with different patterns of abnormal staining as a single group.

(3) Figures 1 and 2 are not shown in the Results section.

In the previous version of the manuscript, Figures 1 and 2 were cited in the Materials and Methods section; however, we agree that it is more appropriate to cite these figures in the Results section. We have made changes to the manuscript accordingly.

Reviewer No. 00068090

(1) To investigate the role of ARID1A gene in primary gastric cancer pathogenesis, real-time quantitative PCR and western blotting should be used to examine the ARID1A expression in paired cancerous and noncancerous tissues. To further investigate the clinicopathological and prognostic roles of ARID1A expression, the authors performed immunohistochemical analyses of the paraffin-embedded gastric cancer tissue blocks.

We would like the reviewer to note that ARID1A is a ubiquitously expressed

protein (ref. 22, Dallas PB, et al. Mol Cell Biol. 2000, 20; 3137-46). In the quantitative PCR and western blotting analysis, the tumor and stromal cells are analyzed together. Therefore, these methods may not properly detect the loss of ARID1A expression in tumor cells. This point is especially true for undifferentiated-type adenocarcinomas, where tumor/stroma ratios can be extremely low. On the other hand, previous studies have shown that a loss or reduction in ARID1A expression is strongly correlated with the presence of *ARID1A* mutations (refs. 19, 22, 23), and immunohistochemistry has been widely used to detect ARID1A inactivation (Chou A, et al. Hum Pathol. 2014, 45; 1697-703, Cho H, et al. Hum Pathol. 2013, 44; 1365-74, Samartzis EP, et. Al. Mod Pathol. 2012, 25; 885-92). Overall, we believe that immunohistochemistry for ARID1A is an appropriate method for detecting the inactivation of ARID1A in human samples.

(2) The authors must show that the loss of ARID1A expression correlated with depth of tumor infiltration and tumor grade, but not only with age, gender, tumor size, distant metastasis and tumor locus or local lymph node metastasis.

As discussed in the manuscript (Table 3, page 19, line 11), many of the clinicopathological features associated with the loss of ARID1A were consistent with those of previous reports (refs. 7, 26, 27). While some differences exist, these differences may be due to differences in the patient populations.

(3) The authors should demonstrate if ARID1A was expressed at different mRNA and protein level in gastric cancer tissues than corresponding non-cancerous mucosa.

As described above, the expression of ARID1A in stromal cells might have compromised the quantitative PCR or western blotting analyses. As already described in the manuscript (page 10, line 15), non-neoplastic cells in the specimens consistently showed diffuse nuclear ARID1A expression in our analysis.

(4) In methods, the authors indicate that sections were deparaffinized and autoclaved at 121°C for 15 min in Target retrieval solution with a high pH of 9 (Dako, Glostrup, Denmark) and then allowed to cool at room temperature. Normally, in immunohistochemistry the slides were boiled in antigen

retrieval buffer containing 0.01 M sodium citrate-hydrochloric acid (pH = 6.0) for 15 min in a microwave oven. What it is the explication to use a solution with high pH?

Antigen retrieval using a high pH buffer is a widely used method (Shi S, et al. J Histochem Cytochem. 1995, 43: 193–201, Pileri SA, et al. J Pathol. 1997, 183: 116-23). We tested both high and low pH buffers for antigen retrieval and found that high pH solution provided consistently better staining results.

(5) The authors should present data about immunohistochemistry quantification. In this sense, the total ARID1A immunostaining score should be calculated as the sum of the percent of positively stained tumor cells and the staining intensity. So, based on the ARID1A expression levels, the gastric cancer patients can be divided into groups.

A previous study has shown that both a loss and a reduction in ARID1A expression suggest the presence of *ARID1A* mutations (ref. 23). Based on this finding, we regarded both of these findings as indicators of ARID1A inactivation. Many previous studies have also used similar classification systems (refs. 7, 23, 26, 27).

(6) The authors in discussion chapter should include that loss of ARID1A expression in cancers may vary depending on tissue types and with microsatellite instability (MSI) status.

As already described in the Discussion section (page 20, line 8), five previous studies have reported a relationship between ARID1A abnormality and MMR deficiency in gastric cancers (ref. 7, 23-26). We have added a description regarding the different mutation prevalences among tumor types.

Reviewer No. 00503442

(1) In order to improve the scientific value of the manuscript several orthographical and grammatical errors found throughout the manuscript should be rectified.

The manuscript has been proofread by a language editor.

(2) Reference no.26 professional English language editing companies should be updated.

We have updated Reference No.26.