Author's response to reviews

Title: Analysis of cell cycle-related proteins in gastric intramucosal differentiated-type cancers based on mucin phenotypes: a new concept of molecular carcinogenesis for early differentiated-type gastric cancers

Authors:

Tamotsu Sugai (tsugai@cocoa.ocn.ne.jp)
Mitsunori Tsukahara (tsugai@iwate-med.ac.jp)
Masaki Endoh (msendo@iwate-med.ac.jp)
Yoshihiro Shioi (y-shioi@mta.biglobe.ne.jp)
Noriko Takebe (tnoriko@iwate-med.ac.jp)
Yoshiharu Mue (yo-mue0427@kih-biglobe.ne.jp)
Hiroo Matsushita (mattsu@mui.biglobe.ne.jp)
Minoru Toyota (mtoyota@sapmed.ac.jp)
Kazuyuki Suzuki (kasuzuki@iwate-med.ac.jp)

Version: 3 Date: 30 December 2009

Author's response to reviews: see over
Dear Dr. Norton:

Thank you very much for your e-mail of December 4, 2009, as well as the comments from the reviewers regarding our manuscript (MS no 1101811677312795). Herewith, I am sending you two revised versions of our manuscript, consisting of 29 pages of text (including 1 page of figure legends), 2 tables and 7 figures. Submitted manuscript has underlined sentences and deleted sentences.

We have incorporated the reviewers' suggestions according to our responses that follow below.

We believe that the revised manuscript addresses all of the major concerns raised by the reviewers and that it is now suitable for publication in Bio-Med Central Gastroenterology. We look forward to your favorable reply. Finally, sentences regarding informed consent and approval of ethics committee have been documented on Page 5, line 5 to 6, according to your suggestion.

Yours sincerely,

Tamotsu Sugai, M.D.
Reply to reviewer 1.

1. Based on your suggestion, we have changed the title “Analysis of cell cycle-related proteins in gastric intramucosal differentiated-type cancers based on mucin phenotypes: a new concept of molecular carcinogenesis for early differentiated-type gastric cancers to “Analysis of cell cycle-related proteins in gastric intramucosal differentiated-type cancers based on mucin phenotypes- a novel hypothesis of early gastric carcinogenesis based on mucin phenotype-”

2. In the present study, immunopositivity for cell cycle-related proteins for tumor tissue was determined by comparing the levels between the tumor tissue and its surrounding non-neoplastic tissue. Reviewer 1 has suggested that the use of an absolute ratio rather than our criteria is meaningful in order to investigate the expression of cell cycle-related proteins. Indeed, separating parameters within the cancers into "low" and "high" categories based on a comparison of these factors with non-neoplastic tissue may be an unconventional method. However, we believe that an immunohistochemical criterion is important for finding the potential activities of cell cycle-related proteins at an early stage of gastric differentiated-type cancers, given that tumor development is significantly influenced by the non-neoplastic tissue surrounding tumor tissue. Therefore, we did not change this criterion in the revised manuscript.

3. Analysis of cdx2 expression has been excluded in the present study based on your suggestion. Some sentences and a paragraph regarding cdx2 have been deleted on Page 6, line 16, page 9, line 14 to line 18 and page 13, line 6 to Page 19, line 1. As a result, related
literature citations (no. 23-26) have been deleted.

**Reply to reviewer 2.**

**Major concerns**

1. As you suggested, gastric phenotype tumors include the same types of cancers based on several combinations of mucin markers. In addition, the intestinal phenotype of tumor cells is also classified into small intestinal and colonic phenotypes. You suggested analyzing clinicopathological differences between gastric phenotypes or small intestinal and colonic phenotypes. However, we could not identify such clinicopathological differences between gastric phenotypes due to the small numbers for this phenotype. On the other hand, although the intestinal phenotype of tumor cells had a large number of cases, in the present study, there were only 18 cases of the colonic phenotype (CD10 negative and MUC2 positive). Therefore, we examined the intestinal type as one group in this study. We found no clinicopathological differences for age, tumor size, location, microscopic type or histological type between the small intestinal and colonic types. One paragraph has been added on page 9, line 13 to 20.

2. This is the first report to extensively analyze gastric differentiated-type intramucosal cancers based on mucin phenotypes. New findings from this study have been provided in Figure 4. In addition, we have provided conclusions on Page 17, last paragraph.

**Minor concerns**

1. We agree with your opinion. Although CD10 is not mucin, immunostaining for CD10 has usually been included in mucin phenotyping.
2. It has been believed among pathologists that the gastric phenotype of gastric cancer shows low-grade atypia in gastric intramucosal differentiated-type cancers. However, in the present study, in the gastric intramucosal differentiated-type cancers that we examined, the gastric phenotype showed high-grade atypia. A previous study showed that a gastric phenotype that was defined based on a mucin phenotype showed high-grade atypia. Although a gastric phenotype showing low-grade atypia resembles gastric foveolar epithelium histologically, in the present study, only one such tumor was observed. It is well known that p53 overexpression is associated with tumor grade. In the present study, we suggest that it is reasonable to show a high frequency of p53 overexpression for the gastric phenotype when compared with other phenotypes. A photograph has been added on page 13, line 14 to page 14, line 2.

3. We agree with your opinion. However, Figure 1 is needed, as it is necessary in order for the readers to understand how to determine the expressions of the markers that we used in this study.

4. “Phenotype” has been added to the beginning of each “mucin”, as you have suggested.

Reply to reviewer 3.

Major concerns

1. We agree with your suggestion. We changed the word “higher” to the word “lower” based on your suggestion.

2. We agree with your suggestion. We changed figure 5 to figure 4 as you suggested.

Minor concerns
1. The histological diagnosis of "intramucosal adenocarcinoma" used by Western pathologists is different from that used by Japanese pathologists, as you suggested. The differences between Japanese and Western pathologists are due to discrepancies in the histological criteria assigned to an intramucosal neoplastic lesion. Western pathologists consider invasion into the lamina propria of the mucosa mandatory for the diagnosis of carcinoma, whereas the preferred criteria among Japanese pathologists are nuclear and structural abnormalities (atypia). In many instances, Western pathologists have diagnosed gastric adenoma or dysplasia in cases where Japanese pathologists diagnosed well-differentiated adenocarcinoma. Therefore, the histological diagnosis of "intramucosal neoplastic lesions" may differ between Japanese and Western pathologists, and even among Japanese pathologists themselves. In particular, the differential diagnosis of small intramucosal neoplastic lesions is very difficult for pathologists. We diagnosed the tumors shown in Figures 1b and 3 as well-differentiated adenocarcinoma based on Japanese criteria. Although the latter (lesion in Figure 3) is considered an intermediate to a high grade atypia of tumor cells by most Japanese pathologists, atypia of the former shows lower, compared with the lesion in Figure 3. As mentioned above, Japanese pathologists regard structural atypia of tumor tissue as important histological criteria. Therefore, Japanese pathologists diagnose the tumor shown in Figure 1 as well-differentiated adenocarcinoma.

2. Description of cdx2 has been deleted based on the suggestion of reviewer #1. We agree with your suggestion. We have added “%” to the vertical axis based on your suggestion.

3. We agree with your suggestion. We have revised some sentences in the legend for Figure 2
on Page 26, line 9 to 13, based on your suggestion.

4. We agree with your opinion. All photographs in Figure 3 are within the same area of the tissue.

Reply to reviewer 4.

1. We agree with your opinion. Cdx2 plays an important role in the development of intestinal epithelia. As you have suggested, immunopositivity for cdx2 was found in the gastric phenotype of tumor cells, although immunonegativity for cdx2 was rarely seen in the intestinal phenotype of tumor cells. However, the role of cdx2 expression in the gastric phenotype of tumor cells remains unknown. Although we are interested in this finding (expression of cdx2 in the gastric phenotype of tumor cells), the description regarding cdx2 expression by differentiated-type intramucosal gastric cancer has been deleted in this text, based on the suggestion of reviewer #1.

2. We agree with your opinion. We prepared higher resolution photomicrographs for Figure 3 with bars, as you suggested.

3. We agree with your suggestion. Although the frequencies for each marker that we examined were shown in Figure 2, the actual % was not indicated in Figure 2. Therefore, the actual % of each marker is necessary in order to understand the results section. We have rearranged the Results section with a slight modification on Page 12, line 9 to 10, based on your suggestion. In addition, a brief explanation of the results for cell cycle-related proteins has been added to the legend for Figure 2.

4. We agree with your opinion. It is well accepted that genetic and epigenetic alterations of tumor
cells are closely associated with early onset of gastric carcinogenesis. Genetic and epigenetic alterations of differentiated-type intramucosal cancer cells are not analyzed in the present study. Although your suggestion may provide a novel point for our study, further study will be necessary to address this issue.