

November 6, 2012

Dear Editorial Office of World Journal of Gastroenterology,

Please find enclosed the edited manuscript in Word format (file name: 353-revised.doc)

**Title:** CDX2 expression and its promoter methylation during metaplasia-dysplasia-carcinoma sequence in Barrett esophagus

**Author:** Kenji Makita, Riko Kitazawa, Shuho Semba, Koto Fujiishi, Miku Nakagawa, Ryuma Haraguchi, Sohei Kitazawa

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 353

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

2 Revision has been made according to the suggestions of the reviewer  
(Reviewer #1)

This approach is interesting in literature where there are few reports concerning epigenetics regulation in Barrett esophagus with metaplasia–dysplasia-carcinoma sequence.

---> We changed paper style into a brief article according to the advice.

(Reviewer #2)

Figure 1: The intestinal metaplasia shown in Figure 1 legend is green (?) in color but blue in the diagram. This needs to be corrected.

“blue” has been changed to “green” in legend for figure 1.

Figure 2: The H&E panel is poorly stained, lacking cellular details. The

authors interpreted that the area shown has IM, HD, and IMC. However, none of the glands have goblet cells in them, making the IM a hard call. Furthermore, the deeply located glands were called HD and IMC but they lack nuclear rounding and stratification needed for the diagnosis of HD. IMC is even more questionable. With the poor resolution for phenotype definition, the findings associated with the claimed phenotypes are unclear.

Figure 3. Again, the resolution and contrast are very poor in panel a.

---> We took the photos by phase-contrast microscopy (Differential interference contrast microscope) for immunostaining. We also used same microscope for HE staining to show the serial sections in a same condition. As pointed by the reviewer #2, HE photos taken by differential interference contrast microscope shows odd-looking and poorer resolution than those by conventional microscopy. We re-took all HE photos by conventional microscopy to match the previous ones.

At least three independent authorized pathologist (Sohei Kitazawa, Riko Kitazawa and Shuho Semba checked the specimens and confirmed the present diagnosis of IM, HD and IMC. Photographs were taken at the boundary of the region to contain various lesions in a single photo and at the same time to show the immunohistochemical expression by the use of serial sections.

Figure 4. Because the areas prior to dissection were not shown, it is impossible to tell what have been dissected (photos for dissected materials were not shown either). Again, this made it difficult to correlate the methylation profiles with pathological diagnoses.

---> These microdissections were done on specimens prestained with immunohistochemistry. Unlike conventional microdissection stained with HE staining, these dissection photos may be less informative but much selective technique. Those areas were chosen by the use of serial HE staining too. The authors really feel sorry for the poor images of the dissection samples, but please understand that these were after

immunostaining and without coverslips.

Thank you very much for these suggestions that have led to the improvement of our manuscript.

3 References and typesetting were corrected

We express our sincere appreciation to the reviewers, Editorial Board, and managing Editor for their suggestions that have led to the improvement of our manuscript. We hope that the revised manuscript will be acceptable for publication in *World Journal of Gastroenterology*.

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

Yours sincerely,

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