

Format for ANSWERING REVIEWERS



June 9, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10357-revised.doc).

Title: Aberrant EphB/ephrin-B expression in experimental gastric lesions and tumor cells

Author: Shintaro Uchiyama, Noritaka Saeki, Kazushige Ogawa

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 10357

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

- (1) As requested by the reviewer (#02457053) or the editor, we have sent the revised manuscript as a "doc" format text file and a "pptx" format figure file.
- (2) Our first manuscript went through English proofreading by a professional English language editing company. We forgot to attach a language certificate letter before. As requested by the reviewer (#02457053) or the editor, we have attached the certificate letter this time (English_editing_certificate_#10357.pdf).
- (3) As requested by the editor, we have described the author's affiliation clearly. This is now on page 1 (words in blue).
- (4) As requested by the reviewer (#02457053) or the editor, we have added the summary (core tip) within 100 words between the Abstract and Introduction section. This is now on page 3 (words and sentences in blue letters) in the revised manuscript.
- (5) As requested by the reviewer (#02457053) or the editor, the blank before or between the references' number was deleted in the revised manuscript.
- (6) As requested by the reviewer (#02457053) or the editor, we have added sections of the Background, Research frontiers, Innovation and breakthroughs, and Applications between the Discussion and References section in the text of the revised manuscript. This is now on page 19-21 (words and sentences in blue letters) in the revised manuscript.
- (7) According the comment of the reviewer (#02457053), we have described the internal control of the RT-PCR analyses in human gastric cancer cell lines. This is now on page 8 and 9 (words and a sentence in blue letters) in the revised manuscript. We used internal controls for the RT-PCR analyses: rat β -actin for expression levels of amplified rat EphBs and ephrin-Bs mRNA in gastric mucosae, and human GAPDH for expression levels of amplified human EphBs and ephrin-Bs mRNA in human gastric cancer cell lines. Because we did not statistically compare expression levels of amplified human EphBs and ephrin-Bs mRNA, we did not describe the internal control in the first manuscript.
- (8) The reviewer (#02905032) commented that measuring semi-quantitative mRNA expression levels by densitometry is old fashioned and the data in figures 1, 3 and 8 should be confirmed by repeating the experiments by using a real-time PCR machine. Still now many data showing mRNA expression levels by semi-quantitative densitometry methods have been published in international journals of high impact factors. If mRNA expression levels examined by "the old fashioned

method” show a significant difference, repeating data by using the real-time PCR machine will also show a significant difference. Therefore our RT-PCR analysis data in figure 3 by the semi-quantitative method do not impair our findings. Moreover we qualitatively showed substantial mRNA expressions of EphBs and ephrin-Bs in figure 1 and 8, and therefore it may be unnecessary to confirm their expression using by a real-time PCR machine.

- (9) The reviewer (#02905032) commented that immunohistochemical data using human gastric tissue samples of gastric ulcer, dysplasia and cancer should be added to the results to underline the reliability of the present findings with the rat experimental models. We think the immunohistochemical study on the human gastric samples is the next and separate study to advance the field of research based on the present findings. EphB and ephrin-B antibodies using the present study are suitable for cryostat sections of rats and mice. In case these antibodies applied on paraffin sections, immunohistochemical reactions do not clearly localized on the basolateral membranes of normal epithelial cells although EphB and ephrin-B are membrane proteins: they usually distribute dispersively in the cytoplasm in paraffin sections even if paraffin sections are treated with antigen activation methods. In this reason we should prepare or make antibodies suitable for paraffin sections of human samples in order to perform those immunohistochemical experiments.
- (10) The reviewer (#02905032) evaluated the English writing of the manuscript and the grade was D. However the manuscript went through English proofreading by a professional English language editing company. Moreover we found two spelling errors (“mayor” and “commonly”) in the comment of this reviewer. This may indicate that the reviewer is not qualified enough for evaluating English writing of scientific articles.

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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