ANSWERING REVIEWERS

June 12, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2936-Review.doc).

Title: Overexpression of miR-196b and HOXA10 characterize a poor-prognosis gastric cancer subtype

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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) There are four independent patient cohorts included in the parts of "Patients and samples", but the author did not introduce group 4.
      ➔ I corrected the mistake from "Four independent patient" to "Three independent patient" in "Patients and samples"of "Materials and methods".
  (2) The full name of HOXA10 (homeobox A10) should appear at the first time.
      ➔ The full name of HOXA10 (homeobox A10) was appeared in "Abstract" and "Introduction".
  (3) Overexpression of HOXA10 related to poor OS was discribed in the manuscript and in Figure 4D, but there were no significant difference between groups (P = 0.202). It is necessary to explain the statistic significance for this result.
      ➔ As p-value of 0.202 is not statistically significant, the sentence was revised to "Overexpression of HOXA10 exhibited a tendency to poor OS": Results, 5th paragraph.
  (4) For figure 5A, it is better to change a figure which was more clearly, and please provide a figure of negative expression of HOXA10 to better represent the result.
      ➔ Figure 5 and figure legends were changed according to reviewer's comment.
  (5) Increased expression of COX2 and cyclin B1 were found in gastric cancer, especially in the C1 group, it is necessary to confirm the results by other methods.
      ➔ I absolutely agree with the reviewer's comment. However, unfortunately, I don't have redundant samples of that group for further experiments. In the future, I will try to perform further validation experiments.
  (6) The clinicopathologic factors of patients with gastric cancer participating in the microarray analysis were listed in table 1, it is necessary to analyse the relationship between miR-196b, HOXA10 and the factors.
      ➔ Table 1 was modified according to reviewer's suggestion. Patients' characteristics were described according to the expression level of miR-196b or HOXA10.
  (7) About the English, please collaborate with a native English speaker to improve language usage.
      ➔ We collaborated with "AJE" for language and editing.
  (8) The current knowledge about HOXA10 in gastric cancer has not been comprehensively
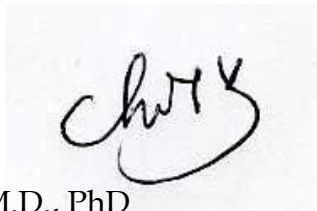
summarized by the authors, because a recently published article (Sentani et al. 2012) was not cited. Besides, the divergent results and the reasons of the divergence should be analyzed.

→ The details were added in "Discussion" according to reviewer's suggestion. "Sentani K, Oue N, Naito Y, Sakamoto N, Anami K, Oo HZ, Uraoka N, Aoyagi K, Sasaki H, Yasui W. Upregulation of HOXA10 in gastric cancer with the intestinal mucin phenotype: reduction during tumor progression and favorable prognosis. Carcinogenesis 2012;33:1081-1088" were added to "References".

(9) The detailed descriptions of staining assessment criteria and the negative control are essential for immunohistochemical staining.

→ Detailed descriptions of staining assessment criteria and the negative control were added in "Method" section. :" Positive expression was defined as staining stronger compared to smooth muscle. Negative expression was defined as staining positivity lower than or similar to smooth muscle.": Materials and methods, 6st paragraph the last sentence.

(10) Multivariate analysis for prognostic factors of gastric cancer patients should be performed.

→ In univariate analysis evaluating stage, miR-196b, HOXA10, histologic type, age, and gender as prognostic factor, stage III/IV (p=0.009) and high miR-196b (p=0.022) were correlated with shorter overall survival. However, multivariate analysis revealed only stage III/IV (0.017) was independent prognostic factor. Therefore, we did not describe the results of multivariate analysis. We concluded that miR-196b might characterize poor-prognosis gastric cancer subtype, but is not yet validated as an independent prognostic factor.

(11) Please detailed clinical importance of the development of personalized cancer therapies in discussion section.

→ Detailed descriptions were added in the last paragraph of "Discussion".

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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