Author's response to reviews

Title: Overexpression of Snail is associated with lymph node metastasis and poor prognosis in patients with gastric cancer

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Author's response to reviews: see over
Author's covering letter for initial submission

Title: Overexpression of Snail is associated with lymph node metastasis and poor prognosis in patients with gastric cancer

Authors:

Version: 1 Date: 13 September 2012

Comments: see over
Dear editor;

We submit a revised manuscript by Shin et al. entitled "Overexpression of Snail is associated with lymph node metastasis and poor prognosis in patients with gastric cancer" in your journal. The manuscript was edited by Edanz as editor’s recommendation. The results obtained from additional experiments have been added and the manuscript has been revised as pointed out by the reviewers. The correction part in description has been highlighted. There is a point-by point description of the changes below.

Reviewer (Jing Gao) comment:
1. The expression level of Snail in cell lines should be detected using Western blot from protein level in Figure 1.
   → The protein level of Snail has been added in Fig. 1 and description has been added in “Results” and “figure legend” (Line 19 of Page 4, Line 10 of Page 10 and Line 5-7 and Line 11-12 of page 28).
2. It seemed that there were conflicting results of Snail expression in Figure 1A and 1B, i.e. in Figure 1A, Snail expressed in SNU216 cells in panel shNT, but in Figure 1B, only a weak band was seen in panel EV in the same cells. The similar problem was existed in SNU484 cells.
   → We repeated this part and data has been changed (Fig.1A and B).
3. The author suggested that Snail maybe a prognosis marker for GC, but the author didn’t describe whether patients received any chemotherapy that could influence the prognosis of GC. The treatment of patients should be analyzed.
   → In our study, patients did not have any preoperative chemotherapy or radiotherapy. We described in the Materials and Methods Section (page 8, line 2)

Reviewer (Tianshu Liu) comment:
1. It would be more convinced if the effect of Lentiviral-based RNA knockdown and overexpression of Snail could be analyzed at a protein level or both mRNA and protein levels.
   → Data has been added in Fig. 1.
2. The authors analyzed the association between overexpression of Snail and the expression of VEGF and MMP11 and came to the conclusion that "Overexpression of Snail was also associated with increased VEGF and MMP11 "(Page 11, Line 20-21) .Why the expression of VEGF and MMP11 were not examined in Snail down-regulated cell line?
   → Although we examined the expression of VEGF and MMP11 in Snail down-regulated cell line, there are no changes in Snail down-regulated cells (sh-snail) compared to control (shNT). Thus we represented the results in Snail overexpressed cells (O/E-snail).
3. In the same respect, why only MMP11 was examined to elucidate the impact of Snail on migration of GC cell lines? MMPs family has many members and to our knowledge, MMP2 and MMP9 are the two most important molecular involved in tumor migration.

→ We also analyzed the mRNA expression levels of MMP2 and MMP9. Interestingly, while mRNA expression of MMP2 and MMP9 were down-regulated in Snail overexpressing cells, they were up-regulated in Snail knockdown cells. We plan to further examine the relation between MMP2 and MMP9, and Snail.

4. As we know, there are wide variations in immunohistochemistry(IHC) analysis for "low" or "high" expression in different types of cancer, and which one should be taken as a criteria is still controversial. In my opinion, it's more reasonable to analyze IHC based on two criteria--intensity and percent positivity. In this manuscript, the authors didn't take intensity into account in IHC analysis, as a result they should cite more evidence to confirm their grading method was superior to others.

→ We agree with reviewer’s opinion about IHC analysis for Snail expression. There are many variations of method to count Snail expression in various type of cancers. We speculate that further extensive multi-institution studies are needed to determine a practical consensus about methods of IHC analysis for Snail expression and cut-off value for Snail overexpression. We described these contentions in Discussion part, Page 14.

5. Expression of candidate genes from cDNA Microarray analysis should be further confirmed in GC cell lines or tissues.

→ We are planning to examine the roles of candidate genes related with Snail overexpression in gastric cancer cells and gastric cancer tissues.

Sincerely yours,

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