

Professor Yuan Qi
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Dear Professor Yuan Qi,

We thank the reviewer for the careful evaluation of our manuscript (Manuscript NO.: 28391) entitled as "HER2-induced metastasis is mediated by AKT/JNK/EMT signaling pathway in gastric cancer".

We agree with the reviewers and tried our best to specially deal with each of the points raised by them. In the revised manuscript, major changes are indicated by highlighting.

The point-by-point responses to the reviewers' concerns are described below.

Again, we highly appreciate the reviewers' constructive comments. We feel that our manuscript has been greatly improved and hope that it is now acceptable for the publication in the *World Journal of Gastroenterology*. We are looking forward to hearing a good news from you.

Thank you.

Sincerely yours,

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Responses to the reviewers' comments

Reviewer 03471272

1. In the Introduction section, the authors should provide the information about the mechanisms of overexpression of HER2 in gastric cancers.

- Since recent studies have reported a high concordance between HER2 protein overexpression in immunohistochemistry and gene amplification by fluorescence in situ hybridization or chromogenic in situ hybridization (Gravalos et al., 2008), HER2 overexpression seems to be directly correlated with HER2 amplification in most cases (Kim et al., 2013) (page 6, paragraph 2, line 10-14).
- **Also, the authors should explain why the efficacy of anti-HER2 treatment of gastric cancer was limited.**
- As the reviewer recommended, we added the explanation for the limited efficacy of anti-HER2 treatment of gastric cancer to the previous sentence: the efficacy of anti-HER2 treatment of GC patients was limited due to intrinsic and acquired drug resistance (page 6, paragraph 2, line 15-17).

2. As the author described, AKT/JNK pathway plays important roles on cell proliferation and survival. Cell proliferation and survival affect the results of cell migration and invasion assays so the authors should provide the data related to cell proliferation and survival of gastric cancer cell lines.

- We agree with the reviewer that cell proliferation and survival affect the results of cell migration and invasion assays.
- Thus, we performed crystal violet assay to analyze a role of JNK/ AKT in cancer cell growth of SNU-216 and NCI-N87 cell lines, which reflects cell proliferation and survival. We added this information in Materials and Methods section (page 12, paragraph 3, line 18-24).
- In the present study, we found that inhibition of JNK/ AKT suppresses cell growth in both HER2-positive gastric cancer cell lines. Thus, JNK/ AKT-induced cell growth of these cell lines might contribute to the results of cell migration and invasion assays observed in the present study. However, both SP600125 and LY294002 treatment enhanced the expression of an epithelial marker E-cadherin and suppressed the expression of mesenchymal markers (Snail, Vimentin and MMP9) in individual gastric cancer cells. Considering that EMT plays a critical role in tumor metastasis, we speculate that JNK/ AKT induces metastatic potential of gastric cancer cells.
- We added our findings in Figure 7 and Results section (page 16, paragraph 4, line 29 and page 17, paragraph 1, line 1-6), and additional sentences in Discussion section (page 19, paragraph 2, line 4-10).

3. In the Results, the authors described that they determined HER2 activation, JNK activation and AKT activation using immunohistochemical techniques. But this procedure of techniques cannot analyze activation. It can only analyze protein expression. The authors should correct their description in the results of immunohistochemical staining.

- We regret that our description was not clear enough to make the reviewer understand our interpretation of immunohistochemical results.
- Although immunohistochemical staining can only analyze protein expression, immunostainings in the present study were performed by using antibodies against active form of JNK (pJNK) and AKT (pAKT), and validation of these antibodies detecting JNK/ AKT activation was confirmed in previous studies (Nam et al., 2013; Choi et al., 2016). In case of HER2 immunostaining, cancer cells with membranous HER2 expression were considered to exhibit HER2 activation as described previously (Kim et al., 2011).

4. I suggest that the text about previous studies be moved from the Discussion section to the Introduction section.

- As the reviewer recommended, we moved the text about previous studies from the Discussion section to the Introduction section (page 7, paragraph 3, line 27-29 and page 8, paragraph 1, line 1).

Reviewer 02725329

1. There is not a sentence about informed consent in the manuscript. Authors should indicate written informed consent from each patient in the study.

- Informed consent from each patient was waived by the Institutional Review Board of Seoul National University College of Medicine because of the following reasons: Most of the patients already died or are not visiting hospital anymore. This study will not cause hazard or exposure of personal information and will be used only for academic purposes (IRB No. 1309-807-522).

References

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Thank you very much!