AUG. 15th, 2016

Dear editor:

RE: "**The impact of homeobox genes in GI cancer**"

Coauthored by **Moon Kyung Joo, Jong-Jae Park, Hoon Jai Chun**

Thank you very much for giving us an opportunity for revision.

A set of comments by the reviewer has proved useful in rewriting this manuscript. We revised this manuscript according to reviewers' comments point by point. We hope that these changes following the reviewers' specific suggestions provide improved quality of manuscript and make this paper more suitable for publication. In our response paragraph, we stressed the changes in the revised manuscript as changing the font to **BOLD.**

Thank you!

Sincerely,

Moon Kyung Joo, M.D., Ph.D.

Jong-Jae Park, M.D., Ph.D.

Hoon Jai Chun, M.D., Ph.D.

## Revision points by points

### Reviewer 1

The strength of the manuscript is in the integration of the findings compiled from a large number of studies. However, the authors could expand their own interpretation of data, point out gaps in some of the work cited, and better explain the conflicting findings. The following suggestions are provided to improve the weaknesses pointed out above:

1) More critical assessment, not the summary, of the work cited is needed. Page 11, for instance: more discussion on how expression of HOXA13 is associated with advanced stage and poor prognosis? What mechanisms could account for this? Page 15, why do they think HOXB13 showed an opposite pattern? Could it be explained by post-translational modifications (Jung et al. 2005)?

**Response)** We agree with your opinion. According to your suggestion, we corrected the following sentences more detail.

; (In page 11 2nd paragraph) Among these genes, upregulation of HOXA13 was associated significantly with ~~tumor size, depth, distant metastasis~~T stage, M stage, advanced UICC stage, histologic differentiation and relapse. ~~and~~Furthermore, patient with positive HOXA13 expression had a lower overall survival and disease-free survival compared with patients with negative HOXA13 expression. ~~poor prognosis~~ The contribution of HOXA13 towards tumorigenesis and aggressive biologic behavior in gastric cancer might be associated with downregulation of tumor growth factor- β (TGF-β) and its downstream target of Runt-related transcription factor 3 by antagonizing Smad3.

; (In page 15 1st paragraph) Previous studies showed that expression pattern of HOXB13 was

site-specific, which was mainly confined to prostate, rectum and distal colon[65], and HOXB13 inhibited the β-catenin/TCF signaling pathway as post-translational manner, which was downregulated in colorectal tumors.

2) Pages 5 and 12, the references cited given don't seem to be relevant to GI cancer.

**Response)** The main contents of page 5 were about general introduction of homeobox gene and cancer, which has been elaborated better in several previous works and reviews of non-GI cancers. The second paragraph of page 12 was about the molecular mechanism for aberrant HOXB7 expression, which has been mainly investigated in other type of cancer cells, including breast, melanoma and cervical cancer, as presented in our manuscript. To our knowledge, only our recent work and Liao et al (Clin Cancer Res 2011;17:3569-78) have suggested MAPK and PI3K/Akt pathways as tentative mechanisms of upregulation of HOXB7 in stomach and colorectal cancers. So we inevitably referred several works which dealt with other type of cancers, and hopefully, reviewer can kindly understand our intention.

3) Pages 11 and 16, statements are vague, and need more elaboration: what relationship and what clinicopathological parameters? Which studies demonstrate the significance to chemoresistance?

**Response)** We agree with your opinion. According to your suggestion, we added several words to expatiate on contents as follows.

; (In page 11 1$^{st}$ paragraph) clinicopathological parameters such as TNM stage, differentiation, overall and disease-free survival rate of gastric cancer patients.

; (In page 16 2nd paragraph) For example, downregulation of HOXA1 under regulation of HOTAIR or miR-100 enhance chemoresistance in pancreas cancer and small cell lung cancer.

4) Pages 10-11, insight on conflicting findings are needed.

**Response)** We understood your comment as the conflicting findings of CDX2 in the process of gastric carcinogenesis. According to your suggestion, we added the following sentence to give our opinion as follow

; (In page 10 1st paragraph) At present, CDX2 appears to be involved in the initiation of the process leading to intestinal type gastric neoplasia such as induction of intestinal metaplasia.

5) The Conclusion section lacks more detailed discussion on the limitations of current knowledge of the impact of homeobox genes in GI cancer.

**Response)** We agree with your opinion. According to your suggestion, we corrected the sentences in the conclusion section as follows.

; (In page 16 1st paragraph) However, intensive understanding of the underlying mechanisms including their transcriptional target genes, and co-factors or downstream effectors of homeobox genes in GI cancers are still lacking. Moreover, current knowledge of the homeobox genes in GI cancer could not reach the clinical efficacy of therapeutic targets or biomarkers, which need to be fulfilled in the future research.

6) In the abstract, the authors indicate that this review will summarize the available research

on homeobox genes in regards to the diagnosis, treatment and prediction of prognosis in GI cancers. However, the clinical relevance is not addressed fully.

**Response)** We agree with your opinion. According to your suggestion, we deleted 'diagnosis, treatment' of the above sentence in the abstract. Because we mentioned the prognostic value of several HOX genes in our manuscript, we remained 'prediction of prognosis' in the sentence.

Finally, I would suggest adding a figure showing key cellular processes involved in up and down regulation homeobox genes of GI cancers to give a quick picture of the findings in the field to date. This would be helpful considering the number of references cited in which epigenetic changes seem to be involved.

While Table 2 is informative, it would be better to see which homeobox expression events are associated with the clinicopathology of the GI tumors: for instance: increased tumor grade or tumor aggression.

**Response)** We agree with your opinion. According to your suggestion we made a new figure presenting the schematic diagram of homeobox genes and their mechanisms which have positive or negative impacts on GI cancers. Please refer our new figure and legend.

Also, we added a footnote at the end of table 2 to summarize which homeobox expression is significantly associated with clnicopathologic parameters including TNM stage and differentiation as follows.

; Note: PROX1, PRRX1, HOXA13 and HOXB7 are associated with advanced TNM stage, while PDX1 is inversely associated; ISX, PROX1, HOXA13 and HOXB7 are associated with undifferentiated type GC.