Author's response to reviews

Title: The gene-reduction effect of chromosomal losses detected in gastric cancers

Authors:

Seung-Jin Hong MD., PhD. (hongsjin@catholic.ac.kr)
Eun-Jung Jeon MD. (jejmd@catholic.ac.kr)
Jung-Hwan Oh MD. (gih@catholic.ac.kr)
Eun-Joo Seo MD. (ejseo@catholic.ac.kr)
Sang-Wook Choi MD. (swchoi2253@catholic.ac.kr)
Mun-Gan Rhyu MD., PhD. (rhyumung@catholic.ac.kr)

Version: 2 Date: 12 October 2010

Author's response to reviews: see over
October 12, 2010

Dear BMC gastroenterology Editor:

Thank you for your reply regarding our manuscript (Manuscript #: 1452757894164081) entitled “The gene-reduction effect of chromosomal losses detected in gastric cancers”. The careful review and helpful comments were of great assistance. Accordingly, we have revised the relationship between the gene-reduction effect of chromosomal losses and the clinical prognosis of gastric cancers. The point-by-point corrections are listed below. The written English has been corrected with the assistance of a professional science writer (http://www.harrisco.net). We hope that the revised manuscript will now be suitable for publication. Thank you for your consideration and we look forward to your reply.

Sincerely yours,

Mun-Gan Rhyu, M.D.

Department of Microbiology, College of Medicine,
The Catholic University of Korea,
505 Banpo-dong Socho-gu, Seoul 137-701, Korea.
Tel: +82.2-2258-7340, Fax: +82.2-596-8969.
E-mail: rhyumung@catholic.ac.kr.

Point-by-point description of the changes made in response to the reviewers’ comments

The similar comments are discussed as follows;

1. REVIEWER 1: Rommel Burbano, Point 1: The authors said in page 6, last paragraph, and
along manuscript that the eight cancer-associated chromosomes examined had a low
density of genes and no stomach-specific genes. So, why those chromosomes were
chosen? The authors should clarify this point.

And REVIEWER 2: Yong Sung Kim, Point 1: This study initially started based on that
the eight chromosomes as those having a low density of genes or the gene-poor
chromosomes and having no stomach-specific genes in line 20 of P6 and line 5 of P18.
Then authors should explain the definition about the gene-poor chromosome. If the
parameter is due to the difference of mean value compared to other chromosomes in
Table 2, is it significant? Can authors present some evidence for the eight chromosomes
having no stomach-specific genes?

In this study, LOH events were examined by five microsatellite marker sets for each
chromosome. Is it possible to estimate overall LOH events on each chromosome with the
small number of MS marker set? So can you say “This indicates that the LOH events tend
to target the chromosomes with a low dose of genes and transcription”? Is there any other
data supporting it?:

We used eight cancer-associated chromosomes that have been found to suffer
frequently from LOH in gastric cancer on previous whole chromosomal LOH analyses [1, 2]. The relationship between the LOH frequency and the clinicopathologic features in
gastric cancers using the same 40 microsatellite marker set on 8 chromosomes were
previously published [3, 4]. We have added new sentences regarding the gene densities of
the cancer-associated chromosomes (lines 14-22 on page 14, “The individual autosomes
were grouped in the order of gene densities. The chromosomes 2, 3, 4, 5, 8, 13 and 18
were categorized into the low-gene-density group of less than five genes per 1 Mb
segment. The chromosomes 11, 16, 17, 19, 20 and 22 had a high density of genes of more
than eight genes per 1 Mb segment. Consequently, of the eight cancer-associated genes
that were examined, six (chromosomes 3, 4, 5, 8, 13 and 18) belonged to the gene-poor chromosomes, and the remaining two genes belonged to the intermediate- (chromosome 9) and high- (chromosome 17) gene-density groups.”).

Although the entire chromosomal arm was not examined in this study, the five microsatellite markers per chromosomal arm with a stable allelic ratio were used to span the entire length of the arm [3, 4]. We have added sentences and references regarding the microsatellite markers on the eight chromosomes analyzed in this study to the Method section (lines 3-10 on page 11, “The highly polymorphic microsatellite markers on 8 cancer-associated chromosomes (3p, 4p, 5q, 8p, 9p, 13q, 17p and 18q), which frequently suffered from LOH in gastric cancer, were used to increase the number of heterozygous alleles on each arm (Figure 1A). The five microsatellite markers on each chromosomal arm showed the clear PCR bands of the heterozygous alleles and spanned the entire length of the eight chromosomes. To ensure the chromosomal reduction, the chromosomal loss was assigned when the LOH event involved more than two microsatellite markers on one chromosomal arm.”).

Other comments are discussed as follows;

REVIEWER 1: Rommel Burbano,

Point 2: The authors describe transcript analyses in the “Results” section, however nothing is mentioned in the “Methods” section. Even if the authors used data from previous papers, it is important to mention this in the “Methods” section:

We have added sentences regarding the transcription analyses to the Method section (lines 1-12 on page 12, “Analysis of in-silico data for the gene density and
transcription of individual chromosomes  A total of 17,723 reference genes identified in a public database (http://genome.ucsc.edu/, March 2006 assembly) were analyzed to calculate the number of genes per 1-Mb nucleotides segment. Serial analysis of gene expression (SAGE) data of normal gastric mucosa was obtained from a public database (http://www.ncbi.nlm.nih.gov/geo/, “SAGE_Stomach_normal_B_antrum”). The transcriptional activity of individual genes was calculated by combining the reference gene map and the expressed gene tags. Based on a comparison of the microarray and SAGE data evaluating the gene expression profiles, the number of transcripts counted in the SAGE data was found to accurately estimate a great difference in the gene activity between the stomach-specific genes and housekeeping genes.

Point 3: The authors cited 116 samples in the “Methods” section, but in the first line of “Results” they cited 118:

We have corrected the sample number (line 4 on page 13, “Most of the gastric cancers (116 out of 145 cases) were...”).

Point 4: The authors should explain the relationship between 17p loss in the intestinal-type cancers and 13q loss in the diffuse-type cancers. Is there any cancer-related gene in the cited regions that could explain that?:

We have added sentences and references regarding to the cancer-related genes on 17p and 13q to the Discussion section (lines 10-15 on page 18, “Chromosome 17, which contained a relatively high density of genes and the P53 gene, was commonly lost in the intestinal- and mixed-type gastric cancers with a high-level LOH. Meanwhile, chromosome 13, which contained a low density of genes and the RB1 gene, was most frequently lost in the
early-onset diffuse-type cancers with a baseline-level LOH (Table 2).”

REVIEWER 2: Yong Sung Kim,

Point 2: It is not clear how LOH-H and LOH-B gastric cancers can be grouped into the high-risk genotypes against the low-risk genotypes of LOH-L and MSI in bottom line of P14 or in middle line of P16. What is a factor to divide gastric tumors into two groups, high-risk or low-risk genotypes? The rational argument should be answered:

We have modified and added sentences regarding to the cancer genotype to the Results section (lines 12-13 on page 15, “The two genotypes related with high-risk-phenotype were...” and lines 21-23 on page 16 to line 1 on page 17, “The LOH-H and diffuse-type LOH-B genotypes were significantly associated with poor clinicopathological features when compared with LOH-L and MSI cases (Table 3).”).

Point 3: In conclusion section, authors insisted that the cell-adverse effect by the gene reduction is more tolerated in intestinal-type gastric cancers than in diffuse-type cancers. In other word, it means that the cell-adverse effect by the gene reduction is less tolerated in LOH-B tumors of diffuse type, although only zero and one chromosomes was lost in diffuse type of gastric cancers. Can you find a diffuse type-specific LOH event? Or could you consider other genetic factor during carcinogenesis of diffuse type of gastric cancer?

We have added new sentences to the Discussion section (lines 6-18 on page 20, “The diffuse-type gastric cancers showed a bimodal distribution for the number of chromosomal losses in consistent with a previous study. In a bimodal distribution, there were one and four chromosomal losses that occur frequently. The high-level LOH induced demethylation might lead to the dedifferentiation of gastric cancer cells that were initially well-differentiated.
Meanwhile, the baseline-level LOH and low-level LOH cases having a few LOH events are likely to preserve the differentiation state formed in cancer progenitor cells. This implies that the histologic type of a given gastric cancer is variously determined according to the adaptive differentiation of cancer progenitor cells as well as the cell-adverse effect of LOH. In the baseline- and low-level LOH gastric cancers that showed a few cases of disease relapse with distant metastasis, the demethylation of CpG-island genes appears to be insufficient to protect cancer cells from the terminal differentiation induced with the overmethylation of the CpG-island genes.”). Also we have modified and added sentences to the Conclusion section (line 7-11 on page 23, “The baseline-level LOH cases with early-onset and diffuse-type cancers recurred in the peritoneal cavity rather than in the distant organs. The LOH events combined with a cell-adverse effect and a dose compensatory response in addition to tumor suppressor gene inactivation may…”).

Point 4: P10: Authors performed the autoradiograph of PCR product to determine the amount of DNA of the tissue lysates or to detect LOH or MSI in tumor DNAs. Then the procedure for radioisotope labeling during the PCR should be described in Materials and Methods section of text in detail:

We have added sentences regarding the procedure for radioisotope labeling in the Method section (lines 13-15 on page 10, “The PCR amplification was performed under a hot-start condition with using a radioisotope (α-32P dCTP, PerkinElmer, Boston, MA, USA) as described previously. Briefly, a...”).

Point 5: P29: in legend of Figure 1, authors defined the extent of chromosomal losses as follows: “Zero and one chromosomal loss was dually classified into low-level (LOH-L) for
the intestinal-type and into the baseline-level (LOH-B) for the diffuse-type.” But in Figure 1B, LOH-L in intestinal type was figured as 0-3 chromosomal losses. It should be defined accurately:

We have modified sentences about the classification of the microsatellite genotype in the legend of Figure 1 (lines 19-20 on page 30, “In cases of diffuse type gastric cancers, zero or one chromosomal loss was classified into the baseline-level (LOH-B).”).

Point 6: P13: in line 3 from bottom, “because most had two or more chromosomes,” chromosomes have to be corrected to “chromosomal losses”:

We have corrected the sentence (line 22 on page 13).

Point 7: P18: In line 12, Table 3 is replaced to Table 2:

We have replaced the sentence (line 15 on page 18).

Point 8: In figure 2: Title on Y axis may be simplified:

We have modified the title on the Y axis in Figure 2 (“Frequency of diffuse-, intestinal- and mixed-type gastric cancers” to “Frequency of gastric cancers”) and the figure legend (line 3-4 on page 31, “The histologic type of gastric cancer was defined as the diffuse- (n = 32, closed box), intestinal- (n = 54, gray box), and mixed-types (n = 44, open box), according to Lauren classification.”).

REVIEWER 3: Jun Yu,

Point 1: As shown in Table 3, in high-risk genotypes, age is positively correlated with LOH level. When it comes to low-risk genotypes, age is negatively correlated with LOH level. This
phenomenon should be discussed accordingly?:

We have added new sentences regarding the MSI genotype cancers to the Discussion section (lines 19-25 on page 20 and 1-5 on page 21, “The MSI-positive cases were oldest among the four genotype groups; baseline-, low-, and high-level of LOH and MSI. Although the gastric cancer patients with low-level and high-level LOH were older than the baseline-level LOH cases that were youngest among the four genotype groups, both the low-level and high-level LOH cases were younger than the MSI-positive cancer patients. The result of this study agrees with the previous studies reporting that the MSI-positive cases were common in late-onset gastric cancers when compared with the LOH-positive cases. The gastric cancer with MSI genotype has been known to undergo the hypermethylation of multiple CpG-island genes involving the mismatch repair gene, which is associated with the aging process. Because the LOH-positive cases may be influenced by the adverse effect of LOH on the cancer progenitor cells, the onset age of gastric cancers is associated distinctly with the LOH and MSI events.”).

Point 2: The age and gender should be included in the models of multivariate analysis, no matter they were significant or not:

We have included the age and gender variables in the models of multivariate analysis (Table 4).

Point 3: Page 8, Paragraph 1, Line 1: “One hundred forty-five” should be “One hundred and forty-five”:

We have corrected the sample number (line 4 on page 8).
Point 4: Page8, Paragraph 1, Line 4: “One hundred sixteen” should be “One hundred and sixteen”:

We have corrected the sample number (line 7 on page 8).

References


