Author's response to reviews

Title: A functional polymorphism in the DNA methyltransferase 3A promoter modifies the susceptibility in gastric cancer but not in esophageal carcinoma

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Author's response to reviews: see over
Dear Editor,

We have revised our manuscript in light of the reviewers' comments and made some required changes to the format of our paper. Now we give a point-by-point description of the changes made in our paper.

**Answers to Reviewer 1 Report:**

Fan et al. showed increased promoter activity of the -448 A allele of the DNMT3A gene in vitro. The allele frequency of -448A among gastric cancer patients and controls was 32.9% and 19.9%, respectively. AA homozygotes show a >6-fold increased susceptibility in gastric cancers but not in esophageal cancers. The authors concluded that -448A is a marker to predict and individual susceptibility to gastric cancers. Although this manuscript potentially contains significant and original findings, there are some points which should be further clarified.

Q1. AG heterozygotes did not show significant increase of the risk of gastric cancers in Table 3. Therefore, one cannot conclude that “AA+AG” is a potential SNP to evaluate the susceptibility even though the p value is 0.001 (Table 3) due to the strong impact of AA homozygotes. Therefore, description about the significance of AG heterozygotes should be toned down or omitted.

**ANSWER:** We have omitted the description about the significance of AG heterozygotes in the risk of gastric cancers in Table 3 and the sentence “AA+AG is a potential SNP to evaluate the susceptibility”.

Q2. The luciferase assay revealed that the promoter activity of the -448 A allele is about twice that of the G allele. The authors should discuss whether the only 2-fold overexpression of DNMT3A may result in alterations of expression levels of tumor-related genes and potentially participate in carcinogenesis or not. In addition, the authors should discuss the reason why the same 2-fold overexpression of DNMT3A does not have impact on esophageal carcinogenesis.

**ANSWER:** We have added some discussion whether more than 2-fold overexpression of DNMT3A may result in alterations of expression levels of tumor-related genes and potentially participate in carcinogenesis in the discussion section of revised manuscript (see discussion section).

In the discussion section, we also added some discussion on why DNMT3A overexpression doesn’t affect the susceptibility of esophageal cancer. "Although it has been reported that several tumor suppressor genes, such as p16, E-cadherin, TIMP3, DLC1 and RUNX3, is common silenced GC and EC, MINT25, RORA, GDNF, ADAM23, PRDM5, MLF1 only showed frequent differential methylation in GC implied there is different genetic and epigenetic mechanism on the development and progression of these two tumor types. In addition, genetic polymorphisms often vary in tissues”. In the meantime, there is different DNMT3A expression of GA genotype carriers in gastric cancer and esophageal cancer patients.

Q3. The author described that they examined immunohistochemically DNMT3A expression
in paraffin sections of gastric cancers (data not shown). Such data should be included in the Methods and Results sections and shown as an appropriate photo. The correlation between DNMT3A expression and AA homozygotes should be examined in gastric and esophageal cancer patients.

**ANSWER:** We have added immunohistochemical DNMT3A expression data in the revised manuscript and this have been described in Methods and Results sections. An appropriate photo has been showed in revised result section. The correlation between DNMT3A expression and G/A genotype have been examined in gastric and esophageal cancer patients and showed in supplementary files.

Q4. The authors should mention the informed consent and approval of institutional review board.

**ANSWER:** We have mentioned the informed consent and approval of institutional review board in the revised Method section.

**Answers to Reviewer 2 Report:**

Q1 The english language has to be improved throughout the paper as an example in the abstract: line 9: promote is promotor, line 12: the distribution were is WAS, line 13: the association were is WAS ...

**ANSWER:** We have modified the manuscript English and corrected some writing errors in it.

Thanks for your consideration.

With best wishes,

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