Author's response to reviews

Title: Genetic Polymorphisms in Osteopontin Promoter Is Associated with the Distant Metastasis of Gastric Cancer and Poor Progress in Chinese Patients

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Author's response to reviews: see over
Re: MS 1802000043673977, “Genetic polymorphisms in osteopontin promoter is associated with the distant metastasis and poor prognosis in patients with gastric cancer”.

Dear Editor,

Thank you very much for your letter and advice. The comments from you and the Reviewers have helped to strengthen this manuscript significantly. We have addressed the comments raised by the Reviewers point-by-point, and the amendments are highlighted in red in the revised manuscript. We hope that this revision is acceptable, and I look forward to hearing from you soon.

Yours sincerely,

Guoxin Zhang, MD

To Reviewer xiaoxiang guan

Major Compulsory Revisions
The manuscript of Fujun Zhao et al. describes a genetic association study of three potentially functional polymorphisms in the OPN genes promoter and the risk of gastric cancer in a Chinese patient population. However, the authors come to the conclusion that variation at nt -443 in the OPN promoter may be associated with the metastasis of gastric cancer. Purpose is risk, then conclusion is metastasis status, so it
seems that this study has a confused aim.

Answer: We thank the Reviewer pointing out this issue. This has been improved in our revised manuscript (Please see line 76-78 in page4 and page 10 on the last paragraph). The aim of this study was to investigate the association between OPN polymorphism and gastric cancer in Chinese population. Finally, we found that the variation at nt -443 in the OPN promoter might increase the risk of distance metastasis of gastric cancer in Chinese population.

Of the 200 patients Helicobacter pylori infection status, 62 patients are seronegative, 138 are seropositive. However, the authors have not evaluated if these two groups of patients differed in the frequency of genotypes, or if the observed association between the selected genotype and survival was restricted to a certain subtype (HP negative/positive) of gastric cancer patients included. Also, as shown in the table 3, the authors should evaluate the frequency of selected variants and TNM stage status.

Answer: We thank the Reviewer for raising this important question. In our study, we showed that the infection rate of Helicobacter pylori was not significantly different between the two groups, and for this reason we did not evaluate the frequency of genotypes or association between survival and a specific subtype (HP negative/positive) in gastric cancer patients. We listed the detailed information about the SNP and TNM stage status in table 3, and evaluated the frequency of selected variants and TNM stage status in table 4.

Although the authors make an effort to do an analysis on the association between selected polymorphisms and metastasis of gastric cancer, the numbers of patients actually are too small to reliably address these issues. For example, as shown in the table3 and 4, for -443 CC genotype, only 1 for Ia, 2 for Ib, 1 forII, 4 for III and 14 for IV. So a well-designed study with larger sample sizes is needed to confirm this finding.

Answer: We thank the Reviewer for pointing out this important issue. We agree that the number of patients is limited in present study, and additional studies are needed
with a larger cohort of patients in order to confirm these findings.

To Reviewer: Hong Fan

- Major Compulsory Revisions

1. In gastric cancer cells, luciferase activities assay showed that C/C genotype has a significantly higher transcription activity than that of T/T genotype. The author mentioned that they found overexpression of OPN in gastric cancer by IHC in previous study. In gastric cancer tissues, whether the polymorphisms of OPN is related to expression of OPN.

   Answer: We thank the Reviewer for raising this important question. Whether the polymorphisms of OPN is related to expression of OPN in cancer patients remain unknown although. Over-expression of OPN was found in gastric cancer samples in a previous study (Gut 2007; 56:782-9). Therefore, additional studies are needed to further elucidate this finding.

2. In result section, the author mentioned that no association was found between the SNPs in the OPN promoter and lymph node metastasis. However, in the abstract, they made a conclusion which is “The variation at nt -443 in the OPN promoter may be associated with the metastasis of gastric cancer. It sounds a little confusing, please explain why.

   Answer: We found that the SNP in the OPN promoter did not increase the incidence of gastric cancer; and the distribution of lymph node metastasis was no significant among three genotypes. But we did find that the SNP in the OPN promoter was associated with the TNM stages of gastric cancer. Besides of lymph node metastasis, TNM Stages included the depth of cancer invasion, metastasis of distant organs and so on. So, the SNP in the OPN promoter could increase the incidence of metastasis
and subsequent death of gastric cancer.

3. SNP should be described as rs. in SNP database instead of nt-443 and so on.
Answer: The rs number for the studied polymorphisms were rs11730582 which were cited the followed article: Chang YS, Kim HJ, Chang J, et al. Elevated circulating level of osteopontin is associated with advanced disease state of non-small cell lung cancer. Lung Cancer 2007; 57:373-80.

-Minor Essential Revisions
1. The author evaluated the association of OPN SNP with gastric cancer with different TNM stage. So larger population should be investigated the frequency of OPN SNP genotype in order to make a unanimous result.
Answer: Thanks for raising the critical issue. We agree that further studies are needed with a large number of patients to confirm these findings. We have included this point in our revised manuscript (Please see page 11, line 219).

Specific comments:
1. Title: The title of this manuscript should be considered carefully and reflect the significant of the present study, or “Genetic polymorphisms in osteopontin promoter is associated with the distant metastasis and poor prognosis in patients with gastric cancer”
Answer: Thank you, we have revised the title “Genetic polymorphisms in the osteopontin promoter increases the risk of distance metastasis and death in Chinese patients with gastric cancer”

2. Key word: The use of comma and semicolon is not uniform.
Answer: Thank you, we have corrected this formatting issue.

Answer: Thank you, we have updated the new reference (Reference #1).
4. Statistical analysis: the author use Student's t-test for comparison of age between cases and controls, is the independent t-test or Paired-Samples T test?

Answer: Yes, we used the independent t-test for comparison of age.

5. References: Lacking Comma between Global and cancer in Reference [1]

Answer: Thank you, this has been corrected.

6. Fig 1: the number of bases in sequences for the same site is not uniform. For example: nt-443TT display AGTTTTCTGAACCTCC; nt-443CT display AAGTTTTCTGAACCTCC; nt-443CC display GTTTTCTGAAC

Answer: We have corrected this in our revised manuscript (in Fig 1).

To Reviewer Venkateshwari Ananthapur

Title

Q: please change the title so as to be more precise and clear.

Answer: As recommended by the Reviewer, we have revised the title as “Genetic polymorphisms in the osteopontin promoter increases the risk of distance metastasis and death in Chinese patients with gastric cancer”.

Abstract

Q1 Abstract has to be rewritten by emphasizing the importance of the study.

Answer: Thanks, the abstract has been revised.

2. It can be written in the past tense form rather than present tense form.

Answer: We thank the reviewer for the suggestion. It has been corrected in the revised manuscript.
3. Number of samples studied was not mentioned in the abstract.
Answer: We have included the number of samples in the abstract in our revised manuscript.

4. Change the sentence” the manuscript must have been ethics”.
Answer: We have changed this sentence in the revised manuscript. (see page 4 and 4 paragraphs)

Introduction
• This section is clear but need to present in proper English.
Answer: We have revised the language problem in the revised manuscript. This manuscript was proof-read by Medjaden Bioscience Limited.

Patients and Methods
1. Did you include all the patients referred to Hospital for the given period. Give details.
Answer: In response to the Reviewer’s concern, we used SPSS v10.0 software to randomly select 200 cases of GC patients from 310 unrelated patients with gastric cancer (the GC group) and used the gender- and age-matched patients to randomly selected 200 controls from the non-GC group (from 591 cases of the control group (the non-GC group). We have included this in our revised manuscript (Please see the last one paragraphs from page 4).

2. Please mention the criteria for the selection of control subjects. Whether they are collected from the same hospital.
Answer: The control subjects must be gender- and age-matched with the cancer subjects. The control subjects were enrolled from the First Affiliated Hospital of Nanjing Medical University and another hospital in Jiangsu Province, China. We have included this in our revised manuscript (Please see the patient information in the revised Methods section on page 5).
3. Methods and statistical analyses were appropriate.

**Answer:** Thank you.

Results

1. Reorient the sentences in the first paragraph as it is not proper.

**Answer:** We have changed the first sentence in the first paragraph.

2. No need of repeating 200 patients and 200 controls in every para.

**Answer:** Thanks, we revised it in the revised manuscript.

3. Presentation style has to be improved.

**Answer:** Thanks. This manuscript was proof-read by Medjaden Bioscience Limited.

Discussion

1. **Rewrite** the discussion by describing the obtained results and then support the same with the earlier literature.

**Answer:** Thank you, we have rewritten the discussion.

2. **The whole** introduction and discussion sections have some disconnected phrases, resulting in an almost incomprehensible text.

**Answer:** Thank you for this comment, we have rewritten the introduction and discussion.

References

References should be written as per journals format.

**Answer:** Many thanks. The references have been modified according to your suggestion.

**To Reviewer Kshitij Srivastava**
Q1: The functional studies for nt -43 have already been reported in previous studies (Schultz et al. and other studies). So the Luciferase reporter assay done in the present study does not add any extra information.

Answer: We thank the reviewer for pointing this out. In the present study, we confirmed that the SNP of nt -443 was a functional site. Schultz et al approved the functional for nt -443 in melanoma cells. And we approved it in gastric cancer cells and in Chinese population, which was the difference between us and Schultz. This will help us in the future studies as well.

Q2: The manuscript has not mentioned some important studies which demonstrate that Osteopontin increases metastasis (Song et al, Brown et al and Ue et al) in gastric cancer.

Answer: Thanks a lot; we have included this information in our revised manuscript (Please see the page 9, line 177).

Minor Essential Revisions

Q1. The rs number for the studied polymorphisms has not been mentioned.

Answer: The rs number for the studied polymorphisms was rs11730582 (nt -443) and rs17524488 (nt -156) which were cited in the following article: Chang YS, Kim HJ, Chang J, et al. Elevated circulating level of osteopontin is associated with advanced disease state of non-small cell lung cancer. Lung Cancer 2007; 57:373-80.

Q2. The manuscript has several grammatical mistakes.

Answer: This has been corrected. This manuscript was already proof-read by Medjaden Bioscience Limited.