Author's response to reviews

Title: Increased expression of MMP9 is involved in poor prognosis in nasopharyngeal carcinoma

Authors:

Bin Wu (wubin1190@126.com)
lixia Li (lisalady@126.com)
zhixiong Yang (yangzhixiong@126.com)
weiren Luo (luoweiren@126.com)
Zhen Liu (narcissus jane@163.com)
weiyi Fang (fangweiyi1975@yahoo.com.cn)

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

We appreciated very much the comments and suggestions made by the Reviewers regarding our manuscript entitled “Increased expression of MMP9 is involved in poor prognosis of nasopharyngeal carcinoma” (manuscript #: 5213987953204447). We have made a thorough revision in English writing for this manuscript. In addition, the following are our responses to the Reviewers’ comments.

1st reviewer:

Comments:

Question 1. Page 4, line 4: Peoples……… before any therapy”. Which methods (RT, chemotherapy with a single agent or more than 1 agents, any adjuvant therapy etc. ?) were used for these NPC patients? Since the overall survival is influenced by the treatment method, it is more appropriate to include this information for data analysis.

Answer:

This is a good question, though by definition a prognostic factor is to predict a patient’s outcome regardless of treatments he or she is going to receive.

As the Reviewer correctly noted, the current standard treatment for NPC include radiotherapy for patients at early stages, or radiotherapy plus chemotherapy for patients at advanced stages. Variations in radiation doses and location, drug selection and dosage, and each patient’s own situation would all have impact on the clinical outcome. As most of other studies in this field, it was impractical to include all of the therapeutic information in this study.

Question 2. Page 5, Results: MMP9 mRNA was highly expressed in NPC tissue: Proteolytic cleavage of the pro-enzyme MMP-9 is required for the activation of MMP-9. What is the rationale for measuring the MMP-9 mRNA? Measuring the expression of MMP-9 proteins using immunohistochemical staining method on
these tissues is more meaningful as the paraffin samples were also analyzed using immunohistochemical staining. Furthermore, the expression level of MMP9 in T1, T2 and T5 was similar to N4 and N5.

Answer:
We agree with the Reviewer that the levels of MMP9 protein in NPC tissues are more biologically relevant to tumor invasion than the levels of MMP9 mRNA. The rational for us to measure the mRNA levels in NPC tissues was because we like to find out if the increase in MMP9 expression occurred at transcriptional level or at post-translational level (i.e. enzymatic activation from pro-MMP9 to active form of MMP9).

Regarding the issue that the levels of MMP9 mRNA in T1, T2, and T5 samples were similar to that in N4 and N5, our explanation is that it was due to the personal variations among those subjects. Nevertheless, the levels of MMP9 mRNA in tumor tissues were generally greater than that in normal tissues.

Question 3. Page 6, line 1 – 3: “We observed …., respectively”. This sentence is not clear.

Answer:  We are sorry that this sentence was actually not clear in expressing what we wanted to say. To avoid any possible confusion, we have removed this sentence from the revised version. This removal would not affect the report of data.

Question 4. Page 6, line 4 – 5: the p value for the MMP9 expression in the normal tissue is p=0.004, indicating that MMP9 is also highly and significantly expressed in the normal tissue. This observation is inconsistence with the statement in page 5, line 20.
Among all 164 NPC patients, 127 (77.4%) cases showed higher MMP9 expression. In comparison, high level expression for MMP9 protein was only 51.5% (17/33) in cytoplasm of normal epithelial cells (p=0.004) (Table 2).

Answer:

Thanks to the Reviewer for pointing out this issue. We had a small error in reporting the number of non-cancerous nasopharyngeal FFPE tissues we used in the study. Instead of the original number of 33, we re-checked this issue and found that we actually used only 32 tissue samples. Subsequently, we performed another round of statistical analysis using $\chi^2$ method, and the result was $P=0.008$. We have made corrections in the revised version of the manuscript.

Question 5. Page 6, line 5 (Table 2): There are 2 Table 2 in the additional file!

Answer: We apologize for the carelessness. We have made corrections in the revised manuscript.

Question 6. Page 6, line 9: Table 1. The order of Table 1 should be before Table 2 (line 5).

Answer: Based on the Reviewer’s suggestion, we made a new table as Table 1, and the original tables have been accordingly changed to Table 2-4.

Question 7. Table 3: multivariate survival analysis of MMP9 protein expression: Data analysis for the age, gender and smoking was not done in this Table.

Answer: The original Table 3 is now changed to Table 4. Because in the univariate analysis, age, gender, and smoking were not significant factors, we chose not to include them in the subsequent multivariate analysis.
**Question 8.** Page 8, 2nd paragraph, line 8-9 “However, no reports concerning the correlation between MMP9 expression and the survivin of NPC patients have been published”. What is the relationship between MMP9 and surviving?

**Answer:**
Again, we apologize for the carelessness. “surviving” should be “survival”. We have corrected it in the revised manuscript.

**Question 9.** Page 12, Figure 2 legend (line 2): where is A1?

**Answer:** We are sorry for the typo. The “A1” should be “A”, and we have corrected it in the revised manuscript.

**Question 10.** The association between high level of MMP2 (but not MMP9) expression and poor survival of NPC patients had previously been reported (Eur. J. Surg. Oncol.,30, 560 - 564). The discrepancy between these 2 studies needs to be discussed. (Wong TS, Kwong DL, Sham JS, Wei WI, Kwong YL, Yuen AP. Clinicopathologic significance of plasma matrix metalloproteinase-2 and -9 levels in patients with undifferentiated nasopharyngeal carcinoma. Eur J Surg Oncol. 2004 Jun;30(5):560-4.

**Answer:**
We appreciate the Reviewer’s input. In Wong et al. study, they reported that the increased levels of plasma pro-MMP9 did not correlate with patients’ clinical outcome. However, in our study, we used NPC tissues rather than plasma tissues to examine the levels of MMP9 protein. Similar to what reported by Wong et al., the levels of MMP9 in NPC tissues were also increased significantly. Moreover, we found that the NPC tissue MMP9 levels did correlate with the survival time of NPC patients. The discrepancy between our data and Wong’s data would be most likely due to the different tissues we used in our respective studies.
Question 11. Typos: e.g.

Front page: The title “……invovled……”; (involved),

Page 4, line 6: …”reserved…” (preserved), and other typos elsewhere in this manuscript.

Page 5, line 10: abbreviation for “NESG”

Answer:

We thank the Reviewer very much for pointing out these errors. We have corrected them in the revised manuscript.
2nd Reviewer's report:
- Major Compulsory Revisions:
  In the current manuscript the authors studied the expression of MMP-9 in NPC and its correlation with clinicopathological parameters of the disease. The manuscript is acceptable and the results interesting.

**Question 1:** Some errors are present in the text such as invovled (title). The manuscript should be checked for typing errors and english spelling improved.

**Answer:**
We like to thank the Reviewer for pointing out the errors. We have made a thorough revision in English writing, and corrected errors in the revised manuscript.

**Question 2:** The authors should indicate the histology of the tumors (undifferentiated, …).

**Answer:**
The histopathology category of all paraffin-embedded NPC samples was undifferentiated.

3-The authors should include in the discussion section the impact of the heterogeneity of nasopharyngeal tissues on mRNA extraction: what is the origin of the MMP-9 mRNA in NP tissues and NPC tissues (epithelial cells, lymphocytes…).

**Answer:**
Immunohistochemistry showed that MMP9 was expressed predominantly in the epithelial cells in both NPC and non-cancerous nasopharyngeal tissues. There was not obvious MMP9 expression in lymphocytes. So we thought that MMP-9 mRNA was also predominantly expressed in the epithelial cells of both NPC and NP tissues.

**Question 4:** Using immunohistochemistry, MMP-9 expression was found predominantly in the cytoplasme and is involved in the destruction of the basal membrane. Since a significant association has been found with N it will be interesting to discuss this result.

**Answer:**
This is a good suggestion. We added a paragraph to discuss the role of MMP9 in N classification.

**Question 5:** The discussion section should be revised and improved. The authors present only the littérature linked to their study and only report their
results at the end of the manuscript (there’s no discussion of the results).

Answer: Based on the Reviewer’s suggestion, we revised the Discussion section.