Peer Review File

<mark>**Reviewer A**</mark>

1. Abstract-no changes

Reply 1: Thank you very much for your hard work.

2. Introduction-"BMP6 is particularly important for iron homeostasis because it has been identified as the most important physiological regulator of "heparin" affecting iron overload"-It should be hepcidin instead of heparin.

Reply 2: Thank you very much for your hard work.
Changes in the text:We have already replaced "heparin" with "hepcidin" in the text and marked it in red font.

3. Methods-No changes

Reply 3:Thank you very much for your hard work.

4. Result and discussion- Kindly use an alternate better term instead of low expression of BMP 6 is associated with "higher malignancy".

Reply 4:Thank you very much for your hard work.
Changes in the text:We have replaced "higher malignancy" with "with poor prognosis in patients" in the text.

5. State limitations of the study

Reply 5:Thank you very much for your hard work.
Changes in the text:We have added the limitations of this article at the end and marked them in red font.

<mark>**Reviewer B**</mark>

Song et al., investigate and suggest a potential role of BMP6 in gastric cancer, associating a positive prognosis to its expression levels.
The experimental plan is well organized, and the major parts of experiments are well performed, but the protocols of the overexpression and silencing of BMP6 in cultured cell experiments are not reported, and it is not possible to evaluate the results.
Data interpretation is well discussed, and the message is clearly stated.
The statistical analysis is sufficiently performed.

The literature cited is appropriate, and the length is commensurate with the message.

For the mentioned reasons, the manuscript may be accepted for publication with major revisions.

Major points:

1) Overexpression and silencing experiments could be described in detail.

Reply 1:Thank you very much for your hard work.

Changes in the text:We have added relevant content to the methodology section, providing a more detailed description of the Overexpression and silencing experiments, and marked it in red font.

Reviewer C

The manuscript attempts to address the lack of understanding of BMP6 expression in gastric cancer and its relevance to malignancy. Whereas the topic is of relevance, I have quite a few remarks:

1. Overall, the language should be proofread by a native speaker. The grammar and flow of text need improvements. The different results are partly described in an unclear manner.

Reply 1: Thank you very much for your hard work.

Changes in the text:We have invited professional native English speakers to edit our manuscript, and the edited content has been marked in red font in the text.

2. The Materials and Method section lacks information about the data extraction method using the mentioned database.

Reply 2: Thank you very much for your hard work.

Changes in the text:We have made additions to the methodology section of the manuscript, providing an explanation of the data extraction methods from the database, and the modified content has been marked in red font.

3. The "Results" chapter is not clearly divided into sub-chapters, lack of clarity.

Reply 2: Thank you very much for your hard work.

Changes in the text:We have rewritten the results section, dividing it into distinct sections for each part, and marked the changes in red font.

Fig. 1: I would like to see at least one image of the actual tissue microarray. What data are generated using the TCGA database and what data using the microarray? The descriptions in the text and in the figure do not match.

Reply 3: Thank you very much for your hard work. Perhaps due to our oversight, some parts of the description were not clear enough.

Changes in the text: We have made modifications to certain parts of the text to enhance clarity. Additionally, we have included relevant immunohistochemistry images in Figure 1c. The original associated image files will be provided in the supplementary materials.

Fig. 1a/b: Are the data used for fig. 1a and 1b the same? If yes, why are there different significances?

Reply 4: Thank you very much for your question. The results represented in Fig. 1a and 1b are both intended to demonstrate the high expression of BMP6 in normal tissues and low expression in tumor tissues. However, the data used for Fig. 1a compares cancer and adjacent tissues, which may not be from the same patient's tissue. In contrast, Fig. 1b requires a paired comparison of cancer and adjacent tissues, typically from the same patient.

Fig. 1c: What statistical test was used to calculate significance? It does not look significant to me just by glancing over. Why are the error bars so small if the sample distribution is clearly quite wide?

Reply 5: Thank you very much for your question. Regarding the statistical methods, we used independent sample t-tests. The small error bars in the figures might have been due to the way the images were created. We have reworked the images to make them appear clearer and more understandable.

Fig. 2c: What does "P23" stand for? Why do we not see any BMP6 expression in the WB?

Reply 6: Thank you very much for your question. "P23" in the text represents the control group, and this may have been unclear due to our oversight. We have added supplementary labeling in red font in the caption of Figure 2 to clarify this. In the experimental methods section, we have also provided additional information. To validate BMP6 overexpression, we detected the flag-tagged protein. When the flag-tagged protein is detected, it indicates that the plasmid overexpressing BMP6 has been successfully transduced into the cells.

Fig. 2d, f, g: The graphs lack proper labelling of the cell lines analysed. How many repeats of this experiment were done? Error bars are very small.

Reply 7: Thank you very much for your question. We have provided detailed labeling and explanations for the cell lines used in Fig. 2d, f, and g. Additionally, we have included supplementary information in the methods section of the manuscript. All experiments were conducted three times, and statistical calculations were performed using independent sample t-tests.

Fig. 2h+I: The colour intensity of the plate photographs and the quantitative graph do not match.

Reply 8:Thank you very much for your question. This might have been due to issues with the image output quality. We have re-exported our images with increased resolution to make them

clearer and more understandable.

Fig. 3e: Why suddenly a different cell line (AGS-7901) which has not even been described in the text or materials and methods?

Reply 9:Thank you very much for your feedback. We have carefully reviewed and found that, due to our mistake, we incorrectly wrote "SGC-7901" as "AGS-7901." This was an error on our part. We have made the necessary corrections in the images. Once again, we appreciate your diligence and attention to detail.

I would have liked to see the same WB on normal non-cancerous gastric cells and cells overexpressing BMP6.
Fig. 3 in general: A chemical inhibition of NFkb signalling would be useful to prove the said hypothesis of BMP6 influencing carcinogenesis via NFkb.

Reply 9: Thank you very much for your suggestion. In our future research, we will conduct experiments to verify BMP6 overexpression in normal cells. Additionally, we plan to explore the relationship between BMP6 and gastric cancer development by inhibiting the NF-κB pathway using inhibitors in our upcoming experiments. We have already included these considerations as limitations in the conclusion section of the article.