Response to Editor and Reviewers

Dear Editor and reviewers:

First and foremost, I would like to express my gratitude for your efforts on reviewing my manuscript entitled "Osteopontin promotes gastric cancer progression via PI3K/AKT/mTOR signaling pathway ".

Thanks to your valuable suggestions, our work has been further improved. After receiving the suggestions, our team reviewed our research and made some additions and modifications according to your suggestions. We have revised our manuscript one by one according to the reviewer's suggestion.

We look forward to publishing our work in your journal at an early date. Thank you for your hard work for us.

Reviewer #1:

Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: Manuscript verified the important role of OPN in gastric cancer in cell growth and migration/invasion in gastric cancer cells. Works nicely showed the relationship of the levels of expression of OPN and cell proliferation, whereas knockdown of OPN suppressed cell proliferation and migration/invasion. Experiments are well designed and results are well presented. Interpretation and conclusion are consistent with their results. The role of OPN in cancer via PI3K/Akt pathway has been well reported in many cancers. This manuscript supports previous studies that OPN also plays important role in gastric cancer. Some comments are as follows.

1.Knockdown experiment in this manuscript was performed by shRNA or siRNA, since shRNA is usually a single strand RNA.

2. In Fig.2A, the image did not reveal anything, please edit to present the comparison between groups of transfection.

3. To make the interpretation more complete about the signaling via PI3K/Akt/mTOR, western blot should be performed with phosphor-mTOR along with other kinases.

Response:

First and foremost, I would like to express my gratitude for your efforts on reviewing my manuscript. Thank you for your recognition of our manuscript, and thank you very much for your valuable suggestions.

1.As you said, shRNA and siRNA are both techniques that interfere with RNA, tools that inhibit protein expression through gene silencing. shRNA needs to be introduced into cells through the

vector, and then siRNA can be obtained by the enzyme digestion mechanism in the cell, and finally exert RNA interference. There is no specific difference in transfection efficiency between the two. The knockdown experiment in our study was carried out using shRNA technique.

2.Fig.2A shows the photos of SGC-7901 cells transfected with OPN-shRNA under fluorescence microscopy. Fig.2BCD shows the comparison of the interference efficiency between groups of transfection in three different sequence-specific OPN shRNAs and negative control shRNA(NC-shRNA). The results showed that OPN-shRNA3 had the highest interference efficiency on OPN, and could be used as a suitable model to determine the effects of OPN knockdown. At the same time, there was no significant difference in expression between control and SGC-7901 cells transfected with NC-shRNA.

3.Thank you reviewers for your valuable suggestions. As you suggest, for a more complete explanation the signaling via PI3K/Akt/mTOR, phosphor-mTOR can be performed with other kinases by western blot. We reviewed the literature and found that the downstream target of PI3K/Akt is mammalian target of rapamycin (mTOR).

We analyzed the protein expression levels of mTOR, AKT, P-AKT of SGC-7901 after they were OPN knockdown or added to PI3K inhibitor (LY294002). The western blotting analysis showed that the P-AKT protein expression level in these two groups was significantly lower than that in the control group. Therefore, we speculated that phosphor-mTOR, as a downstream target of P-AKT, would decrease its protein expression level. Based on the above results, we speculated that OPN promotes proliferation, invasion and migration of GC SGC-7901 cells through PI3K/AKT/mTOR signaling pathway. If possible, we will refine these experiments in the future to better explain the PI3K/Akt/mTOR signaling pathway.

Reviewer #2:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Hello Thank you for interesting manuscript. I have only two points:

1. Please include information from studies in introduction or discussion

^ Di Bartolomeo M, Pietrantonio F, Pellegrinelli A, Martinetti A, Mariani L, Daidone MG, Bajetta E, Pelosi G, de Braud F, Floriani I, Miceli R. Osteopontin, E-cadherin, and β-catenin expression as prognostic biomarkers in patients with radically resected gastric cancer. Gastric Cancer. 2016 Apr;19(2):412-420. doi: 10.1007/s10120-015-0495-y. Epub 2015 Apr 11. PMID: 25862567.

Cao DX, Li ZJ, Jiang XO, Lum YL, Khin E, Lee NP, Wu GH, Luk JM. Osteopontin as potential biomarker and therapeutic target in gastric and liver cancers. World J Gastroenterol. 2012 Aug 14;18(30):3923-30. doi: 10.3748/wjg.v18.i30.3923. PMID: 22912540; PMCID: PMC3419986.

2.Do you perform or planned to perform multivariate mathematical model to prognostic analysis of the osteopontin expression in GC progression? If YES, it will be great to include it in manuscript.

Response:

First and foremost, I would like to express my gratitude for your efforts on reviewing my manuscript.
Thank you for your recognition of our manuscript, and thank you very much for your valuable suggestions.

1.We have carefully read the two articles you provided, and found that they are really good articles that we missed, so they have been cited in the article. (See Reference 15,27).
2.Thank you again for your advice. In the future, we will review the literature and plan to implement multivariate mathematical models to analyze the expression of osteopontin in gastric cancer progression in future studies, and hope to have the opportunity to share our work.

(1) Science editor:

The manuscript has been peer-reviewed, and it' s ready for the first decision.

Response:

Thank the reminder from the science editors, we have adjusted the format and font of the full text, while further language polishing of the manuscript has been carried out to ensure that all errors related to grammar, syntax, format and so on have been resolved to make the revised manuscript meet the publication requirements.

(2) Company editor-in-chief:

I recommend the manuscript to be published in the World Journal of Gastrointestinal Oncology. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/.

Response:

Thank you, editor-in-chief, for recommending our manuscript to be published in the World

Journal of Gastrointestinal Oncology and for your review and suggestion on our manuscript. According to the chief editor's requirements, we visited the Reference Citation Analysis (RCA) recommended by you, and supplemented and improved the highlights of the latest cutting edge research results, so as to further improve the content of the manuscript (for example, reference 15, 27)

Finally， Thank the reviewers for their suggestions on our work again. Because of these suggestions, the revised article get the chance to become better. I appreciate all the help offered by editors and reviewers. If there is any modification need to be conducted, please be sure let me know. Our team is amenable and prepared to do any further adjustment.

With best wishes,

fanjie