October 8, 2021

Subject: Revision and resubmission of manuscript NO.: 70592, Meta-Analysis

Dear editors,

We would like to thank the reviewer for careful and thorough reading of our manuscript "MicroRNAs' prognostic function in patients with osteosarcoma: A meta-analysis" and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript. Below, we also provide a point-by-point response explaining how we have addressed each of the reviewers' comments. We look forward to the outcome of your assessment.

Reviewer #1:

Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: In this manuscript, Gao et al. analyzed microRNAs' prognostic function in patients with osteosarcoma by searching several databases like PubMed, EMBASE, Web of Science and so on. This article strictly followed the steps of meta-analysis. Moreover, authors conducted subgroup analyses of the relationship between miRNA expression and OS to identify potential sources of heterogeneity. Finally, authors concluded that the high expression level of miRNA had a higher risk of poor prognosis of osteosarcoma. However, there are still several points they should revise as follows:

Comment 1: This paper lacks innovation. Similar meta-analysis has been published and there is no more original and deeper analysis seen in your article. Focusing on a specific microRNA to analyze its function in patients with osteosarcoma might be better.

Response: Thank you very much for your comments on our manuscript. All your suggestions are very important to us, both for composing the manuscript and our further research. Based on your opinion, we carefully analyzed the included studies to find specific microRNAs that may be used as prognostic markers of osteosarcoma.

We found that miR-21 is the most studied. There were 3 studies assessing miR-21 as a predictor of overall survival in osteosarcoma. We pooled the HRs using the random-effects model. The results showed that high expression levels of miR-21 were associated with poor OS in osteosarcoma (HR = 2.86, 95% CI 1.20 - 4.53, P = 0.001). In addition, there was one study that accessed the correlation between miR-21 expression and DFS in osteosarcoma, which indicated that high expression levels of miR-2 were associated with poor DFS in osteosarcoma. Therefore, we believe that miR-21 may be used as a marker of poor prognosis in patients with osteosarcoma.

Comment 2: In "Materials and Methods" → "Literature Search strategy": "osteosarcoma" or "osteosarcoma tumor" and "microRNA" or "miRNA" or "miR" and "prognosis" or "prognostic" or "survival" or "outcome" should been modified to ("osteosarcoma" or "osteosarcoma tumor") and ("microRNA" or "miRNA" or "miR") and ("prognosis" or "prognostic" or "survival" or "outcome").

Response: We have modified this section based on your suggestion.

Comment 3: Forest plots should be more clear and beautiful. The gray parts in forest plots should be deleted, or modify them to be more beautiful.

Response: According to your suggestion, we reproduced the forest plot and deleted the gray part, to make the new forest plot look clear and beautiful.

Comment 4: In Discussion, authors only analyzed the relationship between the expression of miRNA and the prognosis of patients with osteosarcoma in a superficial level. Which and how miRNAs affect the prognosis of patients with osteosarcoma should be further analyzed.

Response: Based on your suggestion, we have included in the discussion how miR-21 affects the prognosis of patients with osteosarcoma. MiR-21 has been demonstrated to be a tumor oncogene and plays an important role in tumor progression of various types of tumors. A recent study suggested that, LncRNA neuroblastoma-associated transcript 1 (NBAT1) suppresses the expression of miR-21 and also targets the miR-21-associated genes in osteosarcoma. In addition, miR-21 modulates cell invasion and migration by directly targeting reversion-inducing-cysteine-rich protein with kazal motifs (RECK) and phosphatase and tensin homologue gene (PTEN) in osteosarcoma. Moreover, in osteosarcoma cells miR-21 reduced the anti-tumor effect of cisplatin by regulating Bcl-2 expression.

Reviewer #2:

Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: This manuscript is interesting work about evaluating the role of MicroRNA's as biomarker for osteosarcoma; I have several concern that should be addressed before publication as fellow:

Comment 1: Title is can be more informative and interesting.
Response: We are very grateful for your comments regarding our manuscript. We changed the title based on your comments. The new title is "MicroRNAs as prognostic biomarkers for survival outcome in osteosarcoma: a meta-analysis".

Comment 2: In abstract, methods, results, and conclusion was not appropriate; please describe with more details.

Response: According to your opinion, we have revised the methods, results, and conclusion section of abstract.

Comment 3: Core tip is missing.
Response: Thank you for your reminder, we have added core tip to the revised manuscript.

Comment 4: Please specified the limitations of this study in the last paragraph of the discussion.
Response: The limitations of this meta-analysis are detailed in the last paragraph of the discussion. First, we excluded a small number of studies that lacked original data or could not calculate hazard ratio, which may relatively reduce the convincing power of the results. Secondly, some hazard ratio data are extracted from the survival curve, which may bring errors although tiny. Future research should be based on data directly obtained from the original article if possible. Third, although there is no obvious publication bias in this meta-analysis, positive results are more likely to be published than negative results, which may lead to an overestimation of the association between miRNAs and poor prognosis of osteosarcoma. Fourth, almost all of the 54 articles included are from China, except for one from the United States and

one from Japan. Therefore, the applicability of miRNA as a prognostic indicator of osteosarcoma in other countries and regions is unclear, our results need to be proved by more studies from other regions.

Comment 5: Please revised the conclusion to cover your findings.

Response: Based on your suggestion, we revised the conclusion part in the revised manuscript.

Authors

Best regards