Author's response to reviews

Title: MicroRNA-21 and the Clinical Outcomes of Various Carcinomas: A Systematic Review and Meta-analysis

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Version: 5
Date: 27 September 2014

Author's response to reviews: see over
Dear Editors,

We would like to submit the enclosed revised manuscript entitled *MicroRNA-21 and the Clinical Outcomes of Various Carcinomas: A Systematic Review and Meta-Analysis*, which we wish to be considered for publication in BMC Cancer. No conflict of interest exits in the submission of this manuscript. I would like to declare on behalf of my co-authors that the work described was an original research that has not been published previously. All the authors listed have approved the manuscript that is enclosed.

In this work, we analysis the cancer prognostic value of miRNA-21, which is the newest study to systematically assess the association between them in recent years. We conducted a rigorous study of searching, screening, information extraction and analysis, and found that miRNA-21 is an adverse effect on the prognosis of the tumor. In addition, the heterogeneity was explored carefully and publication bias hadn’t been discovered. Finally, our research gave a certain reference function on prognosis of tumor biomarkers in further studies. Therefore, we respectfully submit this paper and hope it may first be published on BMC Cancer.

We deeply appreciate your consideration of our manuscript, and we look forward to receiving comments from the reviewers. If you have any queries, please don’t hesitate to contact me at the address below.

Thank you and best regards!

Yours sincerely,

Li Jinhui

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Response to the Reviewers’ Comments

Dear Ms. Cherry Battad,

Thank you for your careful and efficient review of our paper, and thank you very much for giving us an opportunity to revise our manuscript titled “MicroRNA-21 and the Clinical Outcomes of Various Carcinomas: A Systematic Review and Meta-analysis (MS: 1965314785134127)”. We would also like to thank the two reviewers for their positive and constructive comments and advice regarding our manuscript.

We have carefully reviewed all of the comments. According to the editor and reviewers’ suggestions, we have made extensive modifications to our manuscript and have provided additional data to make our results more convincing. We have also carefully proofread the article to minimize grammatical and bibliographical errors. In this revised version, changes to our manuscript have been highlighted within the document.

Please find below our description of the revisions made in light of the reviewers’ comments:
RE: reviewer #1 or reviewer #2; AU: the authors

Reviewer #1

RE: The meta-analysis covers studies of different cancer types. It would be very informative to list the cancer types (either in table 1 or in the forest plot).

AU: Thank you for your suggestion. According to your suggestions and after consideration, the cancer types are now supplied in Table 1 of our manuscript (P15, Table 1). This information was highlighted to make this issue more clear and comprehensive. In addition, the subgroup analysis of cancer types is now shown in Table 2 of our manuscript.

RE: Please discuss that the meta-analysis is conducted on the basis of different methods (mostly qPCR, but also microarray and ISH). Other types of studies (e.g. p53 mutation status) may have been conducted in a similar manner which could strengthen the choice of analysis. Is ISH a suitable quantification tool? In this reviewers opinion, it is very suitable for localization analysis but not for quantitative analysis. Studies based on ISH could therefore be excluded.

AU: Thank you for your suggestion. As for ISH, it uses locked nucleic acid (LNA) modified DNA probes and has the potential of being used to evaluate tissue morphology. It can not only detect the target nucleotide sequence for qualitative and quantitative analysis, but it also can reflect the cellular distribution. In addition, ISH technology determines the cellular origin of expression and provides information on expression levels in different tissue compartments and cell populations [Boye Schnack Nielsen, MicroRNA In Situ Hybridization, Methods Mol Biol, 2012]. Its greatest advantage is its use for microscopic examination of the studied tissues because the viral physical status may differ from cell to cell, while the commonly used method of qPCR might be a little sensitive. Therefore, we conducted a subgroup analysis of the detection method and found that heterogeneity still existed in the qPCR group ($I^2=77.2\%$). The sample size was not large enough for the other detection methods, and it is likely not useful to continue the subgroup analysis or meta-regression for these methods. The results are shown in Table 2 (P16).
RE: The follow up time is quite short for some studies (Bovell 2013, Jamieson 2012, Capodanno 2013, Zhi 2010). The authors should consider using a cut-off of minimal accepted follow-up time (In fact many of these studies show reduced HR and the data would look better if these studies were censored out). The difference in follow-up time should be considered and/or discussed. E.g. a different used follow-up time can depend highly on the aggressiveness of tumor subtype.

AU: Thank you for your suggestion. As you noted, factors such as tumour aggressiveness, stage and treatment strategies could have a great influence on the follow-up time as well as the survival period. In the Capodanno (2013) and Bowell (2013) studies, the majority of patients were diagnosed with locally advanced disease. Pancreatic ductal adenocarcinoma (PDAC, Jamieson 2012) and astrocytoma (Zhi 2010), of which grade III and IV constitute 62% of cases, are two of the most aggressive malignancies. The prognosis of these patients is quite poor, which might be the reason why the follow-up times of these studies were relatively short.

According to your suggestion, we excluded the four studies mentioned above and re-ran the analysis. The new pooled HR (HR=2.31, 95%CI, 1.78-2.99) was slightly higher than the primary results (HR=2.27, 95% CI, 1.81-2.86). In addition, the new I^2 also increased slightly (79.0% vs. 76.0%). This change reveals that in our current study, the cut-off of a minimal accepted follow-up time and the exclusion of studies with relatively shorter follow-up might result in a greater heterogeneity as well as increased HR. Therefore, we prefer not to exclude these four studies in the current analysis. It is worth mentioning that we conducted a subgroup analysis for subtype of tumours, which is shown in Table2 (P16). We have also added the information for the different follow-up times for different types of cancers, which is highlighted in the discussion section of the manuscript. We admit that your suggestion is very helpful and instructional, and we will take your suggestion into consideration in our further meta-analysis.

RE: Graphical representation of meta-analysis (forest plot): 31 studies are listed. Where is e.g. Kjaer-Frifeldt 2012? The study mentions either 27 publications or 31 cases. How does the number of 31 studies arise here? And please insert a legend on the x-axis (Hazard ratio).

AU: Thank you for your suggestion. We apologize for this error and have corrected it.
When we reviewed our record, we initially had 30 publications with 36 cases (not 31 cases). When we conducted this meta-analysis, we performed the sensitive analysis to remove the low quality studies and estimate the merging effect value again to obtain more robust and reliable results compared to the previous results. Therefore, 3 papers with 4 studies (including Kjaer-Frifeldt 2012) were removed due to the reason mentioned above. However, we only revised the forest plot, but not the related information in the manuscript and tables. The exact number is 27 publications with 31 cases. We apologize for this careless mistake. We have corrected our manuscript and tables (highlighted).

With regard to your second suggestion “please insert a legend on the x-axis (Hazard ratio)”, we have added the legend for the x-axis (Hazard ratio) on our Figure2 legend.

**RE: Language/writing comments (examples):**

*Do not use Spaces in between parantheses (as in line 60)*

- The miR21 target is maspin (not spelled mapsin) (line 76)
- “The existing prognostic and predictive factors are relatively crude” (line 85) is not scientifically sound writing
- “...independent prognostic factor for poor survival of tumor” (abstract, line 50) is not scientifically sound writing
- Authors should avoid using unscientific phrasing such as: “But this is only part of the story” (p. 10, line 285)
- Do not use abbreviations such as "It didn’t". It should be spelled out: "It did not" (e.g. line 237, line 257, line 258)

**AU:** Thank you for your helpful suggestions. According to your suggestions, we have corrected several language mistakes mentioned above in our previous draft and have highlighted them. In addition, we also employed an English-language editing service to improve the English in the manuscript and make the writing more concise, understandable, and to-the-point.

The examples mentioned above are revised as following (all the modifications are highlighted):

1> We have deleted all of the extra spaces between parentheses.

2> We have changed “mapsin” to “maspin” on line 75.
We agree that the sentence “The existing prognostic and predictive factors are relatively crude” (line 83”) is not scientifically sound writing and changed it to “The existing prognostic and predictive factors still need more proof”.

We agree that ”...independent prognostic factor for poor survival of tumor” (abstract, line 49)” is not scientifically sound writing, so we revised it to “miR-21 might be a considerable prognostic factor for poor survival in cancer patients”.

Thank you for your helpful advice. We have deleted this sentence from our manuscript.

Thank you for your careful review. We will avoid using abbreviations such as ”didn’t” (e.g., line 238, line 258) and have revised all of these words appropriately in the manuscript.

Reviewer #2

RE: It is not clear whether the publications eligible to be included in the analysis provide data on miR-21 expression status also after chemo- and/or radiotherapy treatment of patients (see Mat&Meth, 1st paragraph of section “Definition, Data Extraction and Methodological Assessment”). If so, it is better to separate studies on miR-21 expression in patients that did not receive any chemo- and/or radiotherapy treatments from those that did as:

i) in the cancer patients without any treatment, miR-21 expression status is a direct outcome of cancer per se, therefore reflecting the impact of miR-21 on cancer progression, while,

ii) in the patients that received such treatment(s), miR-21 expression status may be influenced by such treatment(s), therefore possibly reflecting the response in terms of miR-21 expression of the tumor(s) to such treatment(s)

AU: Thank you for pointing out these issues, which are all of great importance to improve the quality of our article. As you mentioned, we agree that it is necessary to make our study selection clearer. First, we limited our selection criteria to the patients who were received surgical resection
without any neoadjuvant therapy. Based on your comments, we checked all of the studies included in our analysis to make sure that none of the cases had received chemotherapy or radiotherapy before surgery. Thus, the miR-21 expression status is a direct outcome of cancer itself and reflects the impact of miR-21 on cancer progression without the influence of any neoadjuvant therapy.

Second, as for the content you mentioned in the first paragraph of the Materials & Methods section “Definition, Data Extraction and Methodological Assessment”, our description might be a little confusing. We intended to make the concept of “overall survival (OS)” more complete, so we gave the explanation that the treatment included surgical excision, chemotherapy or radiotherapy. As an example, surgical excision is not regularly used in the treatment of leukaemia and nasopharyngeal cancer, and definitive chemotherapy, radiotherapy or chemoradiotherapy are the main treatment strategy. In this condition, OS was defined as the interval between chemotherapy or radiotherapy and the death of patients or the last observation. However, in our research, all patients had received surgical treatment without neoadjuvant therapy. Therefore, the possible response of such treatment to miR-21 expression does not exist. To avoid confusion, we have added an explanation (line 124) and deleted the irrelevant explanation in the manuscript (line 126).

RE: English language throughout the text must be carefully attended. Often long sentences are hard to follow. Syntactical, spelling and typographical errors diminish the quality of this work. For example:

i) Abstract, Results section: the “heterogeneity measure index of I²=76.0%, p=0.000”, while in the main Results section p=0.001.

ii) Page 3, Background section: mapsin is probably maspin

iii) miR-21 is typed in various ways throughout the manuscript (text and tables), (miR-21, miRNA-21, Mir-21). A uniform nomenclature should be used

AU: Thank you for your suggestion; we have revised the manuscript extensively. We also employed an English-language editing service to improve the English in the manuscript and make the writing more concise, understandable, and to-the-point.

The examples you addressed above were revised as follows (all the modifications are highlighted):

1> In the Results portion of the Abstract, we apologize for the discrepancy between our
statement that the “heterogeneity measure index of $I^2=76.0\%, p=0.000$”, while in the main Results section the p value is listed as 0.001. The p value should be 0.001, and we have revised the abstract accordingly (line 49).

2> Thank you for your careful review. We have changed “mapsin” to “maspin” in line 75.

3> Thank you for your suggestion. We have revised the manuscript so that “miR-21” is used uniformly throughout the manuscript.

RE: I could not find exact matches of some references in Table 1 with those in the reference list. If the reference list does not necessarily match the references of Table 1, than either a separate list with references of Table 1 must be provided or references in Table 1 must be provided in more detail in order to be tracked by future readers.

AU: Thank you for your careful review. We have added all of the references for each case included in Table 1 (p21) so that more details can be tracked by others.

We have carefully revised the manuscript according to the reviewers' comments and also greatly improved the English language of the original manuscript with the help of a professional editing service. Therefore, we would like to re-submit this revised manuscript to BMC Cancer and hope that it is acceptable for publication in this journal.

Looking forward to hearing from you soon.

Best regards,

Yours Sincerely

Bo Zhang and Wei Zhu