PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	MicroRNA-17 and the prognosis of human carcinomas: a systematic review and meta-analysis
AUTHORS	Huang, Chengzhi; Yu, Mengya; Yao, Xueqing

VERSION 1 – REVIEW

REVIEWER	Donald Sellitti Department of Anatomy, Physiology, and Genetics Uniformed Services University of the Health Sciences School of Medicine 4301 Jones Bridge Rd Bethesda, Maryland, USA 20814
REVIEW RETURNED	20-Jun-2017

GENERAL COMMENTS	This manuscript, by Huang et al, describes a meta-analysis of microRNA-17 (miRNA-17) as a prognostic indicator of human cancer. Data was acquired from seven databases, and after applying various exclusion and inclusion criteria, an original total of 405 articles was reduced to a total of 12 articles included in the meta-analysis. The search criteria are adequately described, and appropriate tests of heterogeneity, publication quality and publication bias were employed. The authors conclude that miR-17 could be a useful prognostic indicator of the outcome of several human cancers. One of the strengths of this paper, as the authors point out, is that it is the first meta-analysis of miRNA-17 as a prognostic marker of human cancer. (An earlier publication by a different group had performed a meta-analysis of this miR-17 as a diagnostic marker). The paper in its present form, however, cannot be considered for publication. Major concerns: 1. The included data appear to be skewed toward results from Asian populations, with Table 1 showing that only two of the twelve included studies (79 our of 1096 patients) were from 'Western' populations. This could be related to their choice of databases for the original selection. The results may or may not apply to all populations equally, but the concentration of data from Chinese populations could limit the applicability of the findings until a larger analyisis containing additional patient populations is completed. [Because of this I questioned whether the design was adequate to answer the research objective]. 2. The subgroup analysis results are confusing. First, it seems that the four parts of Figure 3 (A,B,C,D) are replicates of the same graph. This seems to be in error. Also, I don't understand the significance of the comparison of digestive system cancers with non-digestive system cancers. The 'non-digestive system' cancers cover a wide

	spectrum of cancer types, from immune system through lung and skin – a very diverse group for comparison with cancers of the digestive system. Also, analysis based on detection method (qRT-PCR vs. IF) and ethnic group represent comparisons based on very uneven sets of data (i.e., 11 vs. 1, and 10 vs. 2 separate studies). Are such comparisons valid and meaningful? Other concerns: - Errors in English usage; the MS would benefit from a critical reading/editing by someone well-versed in English. - In part because of language issues, it was difficult to understand some important points that were made in the discussion. e.g., in the discussions of lack of heterogeneity and publication bias at the end of the discussion. As written, the arguments were hard to follow. Overall, the discussion section could have been done a better job at placing the meta-analysis findings in the context of the existing literature on miR-17 and cancer.

REVIEWER	Dr. Peter Igaz MD MSc PhD DSc Semmelweis University, Budapest, Hungary
REVIEW RETURNED	10-Dec-2017

GENERAL COMMENTS	This is an interesting manuscript on the relevance of microRNA-17 in various human malignancies. The authors have presented a detailed meta-analysis and review. The conclusions are adequately drawn. The language of the manuscript requires major revision as there are numerous grammatical and spelling errors in the manuscript. The rationale for the study in the introduction is contradictory, as the authors describe "Of the miR-17 family, recent studies are found that miR-17, functioning as a tumor suppressor, may act as an significant tumor indictor. It is much more complicated in the development of cancer, and the increased expression of miR-17 may help to promote carcinogenesis and cancer progression." Is miR-17 then a tumor suppressor of oncogene? Actually it can be both in different tissues, but this should be clarified. Figure 3 should be split, as the four panels are difficult to interpret.

REVIEWER	Zhiyong Zhang Rutgers University
REVIEW RETURNED	12-Dec-2017

GENERAL COMMENTS	In this study, Chengzhi Huang et al. analyzed 12 previously published papers about miR-17 and reported a system review and meta-analysis of miR-17 in various tumors. Based on their reviews, the authors concluded that miR-17 plays a poor prediction in different human cancers. Overall, this is an interesting study. However, there are many shortcomings about the conclusions from this manuscript. Major points: 1, Although many studies reported that miR-17 promotes the cancer progression, like many other miRNAs, in several cancers, miR-17 also inhibits cancer developments. Moreover, the authors obviously did not consider the subtypes' different effects of miR-17, such as miR-17-5p or 3p on the cancers. 2, There are too many grammar mistakes in this manuscript. I suggest that the authors should correct all of this errors before the submission.

REVIEWER	John Ohrvik Center for Clinical Research, Uppsala University, Sweden
REVIEW RETURNED	21-Jan-2018

GENERAL COMMENTS	This interesting meta-analysis studies the relation between microRNA-17 status and the following clinical outcomes; overall survival, disease-free survival, and recurrence–free survival in cancer patients. From a statistical point of view, I have the following comments: 1. The authors need to better explain how they performed Duval and Tweedie's Trim and Fill procedure and add the imputed studies to Figure 5. I also suggest that the authors add the publication bias corrected HR with 95% confidence interval to Figure 2 and both uncorrected and corrected HR with 95% confidence interval to Figure 5. 2. The power of Cochran's Q test is low when the number of studies is small. Higgins' I^2 statistic measures the percentage of variation across studies that is due to heterogeneity. There is an exact relation between Higgins' I^2 and Cochran's Q: I^2 = 100% x [Q-(No of studies -1)]/Q. Unlike Q it does not inherently depend on the number of studies. A confidence interval for I^2 can be constructed using the iterative non-central chi-squared distribution method described in Hedges LV, Pigott TD. The power of statistical tests in meta-analysis, Psychological Methods 2001;6:203-217. I suggest that the authors give a confidence interval for I^2 instead of a P-value for Q in Table 2. A confidence interval will better reflect the low power. 3. Due to the low power of the Q-test I don't agree with the author's conclusion on page 18 line 35 that the heterogeneity is low. I suggest that the authors also assess a random effect model and compare the outcomes of the fixed effect and random effect models. 4. The random effect model used in case of significant heterogeneity has to be better explained. In general random effect models are not a cure for the difficulty to generalize the results of a meta analysis in case of heterogeneity. General comments: 1 Throughout the language has to be improved. 2 Page 13, line 52 implantations. I think the authors mean implementation.

	3 On page 14 line 19 the authors introduce the abbreviation miRNA for microRNA. Thereafter they should use the abbreviation and not switch between microRNA and miRNA. 4 Page 14 line 20 nt is superfluous. 5 Page 17 line 43-45 says that human digestive system cancers remained for further detailed screening. This is not true since in the final selection also human non-digestive cancer studies were included. 6 Figure 2D and Table 2 shows a large difference between the Spanish and the Brazilian study. The authors mention the different assay used (qRT-PCR vs HAS) as a possible reason. Have the authors checked that no Hispanics were included in the Brazilian study? That could also explain the heterogeneity?

VERSION 1 – AUTHOR RESPONSE

Response to Reviewers:
Editor's Comments:
Comment 1: In the introduction, authors say that mIR-17 is considered as a "tumor indictor". I have never heard of that terminology, they should clarify what is the meaning but it seems clear that they want to refer to the opposite of oncosuppressor. Like reviewer Igaz, I was confused about the rationale of the study since the introduction leaves us confused as to whether miR-17 is an oncogene or an oncosuppressor.
Response: We absolutely agree with the editor that "tumor indicator" was wrong. In the Introduction of the revised manuscript we have clarified the role of miR-17 which, as is amply described in the literature for miR-17 as well as other miRNAs, depends on the cellular context meaning it can act as a tumor suppressor or as an oncogene. We hope that the revised Introduction clearly presents the rationale of the study.
Comment 2: The search strategy is dominated by Chinese databases so I also agree with reviewer Sellitti's major concerns regarding the predominance of Chinese studies as well as the questionable validity of the comparisons in the subgroup analyses.
Response: Our meta-analysis included searches from the most widely used authentic databases, Pubmed, Embase, and Web of Science using English language as a filter. We agree that most of the studies reported are of the Chinese population. We have commented on this that further studies are needed to draw any conclusions in other populations.
Comment 3:Since I don't think miR-17 is investigated in routine practice I think authors need to acknowledge this is a preliminary study and that there are no clear immediate clinical implications.
Response: Following your very valid comment, we have clearly stated that our meta-analysis only suggests the clinical potential of miR-17 and has no current clinical implications
Comment 4: Please ensure that your manuscript is thoroughly proofread by a native English speaker prior to resubmission, to check for errors in language. Please include an example of the full search strategy for the study, as a supplementary file.
Response: In response to the Editorial and Reviewers' comments, we have sought assistance from a professional editorial service. Our manuscript is now much improved for grammar and readability. As required, the full search strategy of the study is presented in Supplementary Table 1.

We would also like to point out here that we have revised the authors' information in the manuscript: According to the requirements of our institute, the authors' affiliation should be Department of General Surgery, Guangdong General Hospital (Guangdong Academy of Medical Sciences)and the order of authors' institution (1 and 2) should also be corrected

Response to the Reviewers'Comments:
Reviewer 1
Comment 1: The included data appear to be skewed toward results from Asian populations, with Table 1 showing that only two of the twelve included studies (79 out of 1096 patients) were from 'Western' populations. This could be related to their choice of databases for the original selection. The results may or may not apply to all populations equally, but the concentration of data from Chinese populations could limit the applicability of the findings until a larger analysis containing additional patient populations is completed. [Because of this I questioned whether the design was adequate to answer the research objective].
Response: As stated in our response to the Editor's Comment 2, our choice of databases included authentic most commonly used databases. The Reviewer is factually correct that there was a preponderance of Chinese populations in the reported studies. We have pointed it out in the revised manuscript that the results may have limited applicability to other populations and the need for more data on Caucasians. The detailed search strategy is updated in Supplementary Figure 1.
Comment 2: The subgroup analysis results are confusing. First, it seems that the four parts of Figure 3 (A, B, C, D) are replicates of the same graph. This seems to be in error. Also, I don't understand the significance of the comparison of digestive system cancers with non-digestive system cancers. The 'non-digestive system' cancers cover a wide spectrum of cancer types, from immune system through lung and skin – a very diverse group for comparison with cancers of the digestive system. Also, analysis based on detection method (qRT-PCR vs. IF) and ethnic group represent comparisons based on very uneven sets of data (i.e., 11 vs. 1, and 10 vs. 2 separate studies). Are such comparisons valid and meaningful?
Response: We truly appreciate the Reviewer's comment and have corrected the embarrassing error in Figure 3. We have also corrected the subgroup analysis of disease types consisting of cancers of digestive system, respiratory system, hematopoietic, glioma and osteosarcoma. Detailed information is provided in Table 2 and Figure 3. The analysis of cancer types, detection methods, and ethnic groupswere based on the retrieved studies. The Reviewer is absolutely correct that due to the limited number and uneven types of studies, the power of subgroup analysis may not be reliable. Following Dr. John Ohrvik's advice, we have provided the confidence interval for I2 instead of a P-value for Q in Table 2 which better reflects the low power of Cochran's Q test.
Comment 3: Errors in English usage; the MS would benefit from a critical reading/editing by someone well-versed in English. In part because of language issues, it was difficult to understand some important points that were made in the discussion. e.g., in the discussions of lack of heterogeneity and publication bias at the end of the discussion. As written, the arguments were hard to follow. Overall, the discussion section could have been done a better job at placing the meta-analysis findings in the context of the existing literature on miR-17 and cancer.
Response: Again, we are highly appreciative of the Reviewer's extremely valuable comments. We have addressed every single point raised by the Reviewer. The language has been polished by an editorial service. We do hope that the manuscript is better understandable now and the points we tried to make are easier to follow. We have revised the discussion, cited the relevant references on miR-7 which are supportive of our findings in the meta-analysis.

Reviewer 2
Comment 1: "Of the miR-17 family, recent studies are found that miR-17, functioning as a tumor suppressor, may act as a significant tumor indictor. It is much more complicated in the development of cancer, and the increased expression of miR-17 may help to promote carcinogenesis and cancer

progression." Is miR-17 then a tumor suppressor of oncogene? Actually, it can be both in different tissues, but this should be clarified.
Response: We regret the use of poor language and hard to understand statements. The Reviewer is absolutely correct that miR-17, and for that matter any miRNA can function either an oncogene or a tumor suppressor gene. We have clarified this both in Introduction and Discussion sections.
Comment 2: Figure 3 should be split, as the four panels are difficult to interpret.
Response: As correctly pointed out by the Reviewer, Figure 3 was flawed. We apologize for the error and have corrected Figure 3 in the revised version.

Reviewer 3
Comment 1: Although many studies reported that miR-17 promotes the cancer progression, like many other miRNAs, in several cancers, miR-17 also inhibits cancer developments. Moreover, the authors obviously did not consider the subtypes' different effects of miR-17, such as miR-17-5p or 3p on the cancers.
Response:We are grateful to the Reviewer for very valuable comments. As we have stated in response to the comments by the Editor as well as other Reviewers, in the revised manuscript, we have very clearly stated that miR-17, like other miRNAs, can inhibit or promote cancer development. As for miR-17-5p and -3p, both are located within miR-17 with a stem-loop structure. We did perform the subgroup analysis (Figure 3E and Table 2) and found no significant difference.
Comment 2:There are too many grammar mistakes in this manuscript. I suggest that the authors should correct all of this errors before the submission.
Response: We thank the Reviewer for a very justified criticism. We have sought professional assistance for improving the language.

Reviewer 4
Comment 1: The authors need to better explain how they performed Duval and Tweedie's Trim and Fill procedure and add the imputed studies to Figure 5. I also suggest that the authors add the publication bias corrected HR with 95% confidence interval to Figure 2 and both uncorrected and corrected HR with 95% confidence interval to Figure 5.
Response: We performed Duval and Tweedie's Trim and Fill procedure by using the Stata software and mentioned it in the statistical analysis section. The funnel plot of adjust pool HRs after the analysis of the Trim and Fill method is presented in the Supplementary Figure 1. The altered HR after applying Trim and Fill method was 1.34, 95% CI=1.24-1.46, P<0.001, which was significantly different from the pooled HR (HR=1.45, 95%CI=1.29-1.63, P<0.001). Due to the limitation of current statistical software and insignificant difference between adjust pooled HR and original HR, we have not added the additional data to Figure 2 and Figure 5.
Comment 2: The power of Cochran's Q test is low when the number of studies is small. Higgins' I2 statistic measures the percentage of variation across studies that is due to heterogeneity. There is an exact relation between Higgins' I2 and Cochran's Q: I2 = 100% x [Q-(No of studies -1)]/Q. Unlike Q it does not inherently depend on the number of studies. A confidence interval for I2 can be constructed using the iterative non-central chi-squared distribution method described in Hedges LV, Pigott TD. The power of statistical tests in meta- analysis, Psychological Methods 2001;6:203-217. I suggest that the authors give a confidence interval for I2 instead of a P-value for Q in Table 2. A confidence interval will better reflect the low power.
Response: We are indebted to the Reviewer for an extremely valuable and well-described suggestion. We have followed the suggestion and added the confidence interval for I2 in Table 2, which may better reflect the low power and the potential heterogeneity.
Comment 3: Due to the low power of the Q-test I don't agree with the author's conclusion on page 18 line 35 that the heterogeneity is low. I suggest that the authors also assess a random effect model and compare the outcomes of the fixed effect and random effect models.
Response: We thank the Reviewer for a very relevant and valuable comment. As suggested by the Reviewer, because of the low statistical power of Q-test in view of the limited number of studies

included in the meta-analysis, we conducted random effect analysis on the OS which was similar to analysis of fixed effect model. We have presented this in detail in the Discussion (Please see page 17)
Comment 4: The random effect model used in case of significant heterogeneity has to be better explained. In general, random effect models are not a cure for the difficulty to generalize the results of a meta-analysis in case of heterogeneity.
Response: The heterogeneity may be the result of limited number of studies and samples in our meta-analysis. To address this issue, we have also followed the Reviewer's suggestion in Comment3. We have described the random effect analysis and the reason for using it in Discussion on page 10.
Comment 5: Throughout the language has to be improved.
Response: We have had the manuscript polished by a professional editorial service.
Comment 6: Page 13, line 52 implantations. I think the authors mean implementation.
Response: We are sorry for the oversight and have corrected the error.
Comment 7: On page 14 line 19 the authors introduce the abbreviation miRNA for microRNA. Thereafter they should use the abbreviation and not switch between microRNA and miRNA.
Response: In the revised manuscript, we have consistently used miRNA after describing the abbreviation once.
Comment 8: Page 14 line 20 nt is superfluous.
Response: The letters "nt" have been deleted.
Comment 9: Page 17 line 43-45 says that human digestive system cancers remained for further detailed screening. This is not true since in the final selection also human non-digestive cancer studies were included.
Response: In the revised manuscript, we corrected the subgroup analysis which includes cancers from digestive system, respiratory system, blood, glioma, and osteosarcoma. Detailed information is provided in Table 2 and Figure 3.
Comment 10: Figure 2D and Table 2 shows a large difference between the Spanish and the Brazilian study. The authors mention the different assay used (qRT-PCR vs HAS) as a possible reason. Have the authors checked that no Hispanics were included in the Brazilian study? That could also explain the heterogeneity?
Response: We have tried to better explain the possible reason for the heterogeneity in the Discussion of the revised manuscript (page 11). (Also, please see our response to Comment 3)

VERSION 2 – REVIEW

REVIEWER	Dr. Peter Igaz MD MSc PhD DSc Semmelweis University, Budapest, Hungary
REVIEW RETURNED	03-Mar-2018

GENERAL COMMENTS	My comments have been adequately addressed, the manuscript has considerably improved. I propose its acceptance. P.S. The authors could have made the job of reviewers' much easier if the modifications would be highlighted.

REVIEWER	Zhiyong Zhang Surgery Department, Rutgers University
REVIEW RETURNED	14-Mar-2018

GENERAL COMMENTS	The authors already answered my comments. If the specialist review is finished, I recommend to accept the manuscript.

REVIEWER	John Ohrvik Uppsala University, Sweden

REVIEW RETURNED	15-Mar-2018

GENERAL COMMENTS	The manuscript has improved and the comments and questions have been addressed in a satisfactory manner. I have only one minor comment: (i) Since both the Spanish study and the Brazilian study recruited Caucasians that would rather decrease the heterogeneity compared to having included also Hispanics in the Brazilian study. Please clarify in the manuscript.

VERSION 2 – AUTHOR RESPONSE

Response to Reviewers' Comments:

Reviewer 2

Comment: My comments have been adequately addressed, the manuscript has considerably improved. I propose its acceptance.

Response: We are pleased that the Reviewer is satisfied with our revised manuscript and express our gratitude for the Reviewer's positive recommendation.

Reviewer 3

Comment: The authors already answered my comments. If the specialist review is finished, I recommend to accept the manuscript.

Response: We would like to thank the Reviewer for recommending acceptance of our manuscript.

Reviewer 4

Comment 1: The manuscript has improved and the comments and questions have been addressed in a satisfactory manner.

Response: Thank you for your valuable comment.

Comment 2: Since both the Spanish study and the Brazilian study recruited Caucasians that would rather decrease the heterogeneity compared to having included also Hispanics in the Brazilian study. Please clarify in the manuscript.

Response: We thank the Reviewer for the comment and have included the sentence in the modified version.