Author’s response to reviews

Title: Expression and role of oncogenic miRNA-224 in esophageal squamous cell carcinoma

Authors:

Xiaoyan He Ms (xiaoyanhe1988@126.com)
Zhimei Zhang Ms (1365360153@qq.com)
Ming Li Mr (limingibm@sina.com)
Shuo Li Ms (lishuo1989@163.com)
Lihua Ren Ms (renlihuaxy@126.com)
Hong Zhu Mr (zhuhong1059@126.com)
Bin Xiao Mr (lancent@gmail.com)
Ruihua Shi Mr (ruihuashi@126.com)

Version: 3 Date: 2 July 2015

Author’s response to reviews: see over
RE: Resubmission of our manuscript (#1812497771146365)

Dear Ms. Bravo,

Thank you very much for your email with encouraging news regarding our manuscript. We also thank the reviewers for their positive/constructive comments and suggestions, which truly helped us to improve our manuscript. After incorporating their comments into the revised manuscript, I would like to re-submit it for your consideration for publishing in *BMC Cancer*. The amendments are highlighted in red in the revised manuscript, and our point-by-point answers to the reviewers’ comments are attached below. This manuscript has been edited and proofread by Medjaden Bioscience Limited (Hong Kong, China).

Thank you again, and I hope that the revision is acceptable. I am looking forward to hearing from you soon.

Sincerely,

Ruihua Shi, MD
Department of Gastroenterology
Zhongda Hospital, Southeast University
87 Dingjiaqiao Road, Nanjing 210009, China
Tel.: +86-25-83674636
E-mail: ruihuashi@126.com

Our responses to the reviewers’ comments:

Reviewer: tony E. Godfrey

**Major points**
1. It is unfortunate that the qPCR primer sequences are not available. Since this paper focuses entirely on expression of miR-224 these sequences are critical to the study. Not including them in the manuscript negates the ability of other researchers to independently validate the data in full. Furthermore, many
miRNA's differ from each other by only a few bases. Specificity of the assays is a major concern (particularly with SYBR Green detection) and this cannot be evaluated without the primer sequences being provided. Indeed, even the authors themselves do not know for sure what they are detecting in the study.

We fully understand the reviewer’s concern and agree. We have contacted the RiboBio company staff again for asking the sequences as soon as we received reviewer’s comments. However, they insisted that the sequences could not be disclosed. The technician of RiboBio company explains as follows: Firstly, the primer sets of miRNAs are commercial reagent, which can be purchased at any time if other researchers want to validate the data independently. Secondly, other companies such as Qiagen and Applied Biosystems also do not provide sequences to their customers (due to their intellectual property and commercial interest).

Using online tools, including miRBase and TargetScan, we found that the sequence of miR-224 was very special, which was not close to that of other known miRNAs. Moreover, melting curve analysis of qPCR showed that the specificity of our primer sets of miR-224 was very high. Thus, please don't worry about the specificity of the primers.

2. The statement "The current study demonstrated that miR-224 acts as an oncogenic miRNA in ESCC by targeting PHLPP1 and PHLPP2" is still an overinterpretation of the data. The data supports that mir 224 is oncogenic and that it targets these genes but it does NOT demonstrate that the oncogenic activity is driven by PHLPP1 and 2. A more accurate statement would be, "The current study demonstrated that miR-224 acts as an oncogenic miRNA in ESCC, possibly by targeting PHLPP1 and PHLPP2."

We fully understand the reviewer’s concern and agree. We have revised the conclusion in the manuscript according to the reviewer’s comment (Page 3, Line 20-21; Page 18, Line 16-17).

Minor revisions

1. Thank you for clarifying qRT-PCR data presentation and analysis. I still believe however that figure 1A is very hard to interpret. I suggest that the authors consider re-plotting this using actual expression data but with a log scale on the Y-axis. Statistical analyses can still be performed and reported on transformed data but the figure will be easier to interpret this way.

Thank you for your insightful suggestion. We have revised them accordingly (Figure 1A). Now, the data (the relative miR-224 expression, calculated using $2^{\Delta(\text{Ct of U6})}$) shown in Figure 1A were skewed distribution. Thus, the horizontal lines in Figure 1A represented the median, rather than the mean. A statement have been added in the Figure legends of the revised manuscript (Page 22, Line 8-13) to address this issue.

2. I believe that the use of the term Pathologic Grade (instead of pathologic stage)
throughout the manuscript, tables and figures would help clarify the difference between the pathologic TNM and Pathologic Stage analyses. Pathologic stage is equivalent to overall stage based on TNM, hence my original confusion. Also, in tables and figures pathologic grade should be listed as GI, GII etc as additional clarification.

We fully agree and have revised the text accordingly. The term pathologic stage was displaced by pathologic grade throughout the manuscript, tables and figures. Also, pathologic grade have been listed as GI, GII etc (Table 2, Figure 1C).

The authors may need to improve English throughout the manuscript.

We thank the reviewer for this comment. The revised manuscript has been edited and proofread by Medjaden Bioscience Limited (Hong Kong, China).