Author’s response to reviews

Title: Overexpression of members of the microRNA-183 family is a risk factor for lung cancer: a case control study

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Author's response to reviews: see over
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Dr. Dan Rolf
Editor, BioMed Central

Regarding MS: 1612078295563430, Overexpression of members of the microRNA-183 family is a risk factor for lung cancer: a case control study

Dear Dr. Dan Rolf,

We want to thank you and the reviewers for the careful review of our manuscript. The reviewer’s comments have helped us strengthen this work. Below, we address each and every one of the reviewer’s comments in the hope that the revised manuscript will be satisfactory for publication in your journal. Please note that the amendments are highlighted in red in the revised manuscript.

We hope that this revision is acceptable for publication in your journal and I look forward to hearing from you.

Yours sincerely,

Wangyu Zhu, MD
The answers for reviewers:

Reviewer: Carmen Marsit

1) The authors indicate that the miRNAs selected for examination were chosen based on "magnitude of fold changes and probability values". This statement is vague and not supported by any data presented in the paper. The authors should provide more background on the results of the microarray and elaborate on the method of selecting the miRNAs to be studied. While the authors mention that they used SAM to determine differentially expressed miRNAs at a cut-off of P<0.05, there is no mention of correction for multiple comparisons or FDR adjustment, a requisite for microarray analysis. Additionally, the authors could consider providing a heatmap of the microarray results as supplementary data.

Reply: We have provided the fold changes and P-values for miR-96, miR-182 and miR-183 in the microarray analysis, and multiple comparisons have also been included in the revised manuscript (see page 10 in the first paragraph of Results). In addition, the heat map of the microarray results was shown in our recent study (Liu X, Zhu W, Huang Y, Ma L, Zhou S, Wang Y, Zeng F, Zhou J, Zhang Y: High expression of serum miR-21 and tumor miR-200c associated with poor prognosis in patients with lung cancer. Med Oncol 2011, Apr 24. [Epub ahead of print]).

2) While the Mann-Whitney U-test is an appropriate non-parametric test to look for associations between miRNA expression and covariates of interest, the authors should follow these analyses up with linear or logistic regression models to assess these associations while controlling for confounders. Survival analyses should also control for confounders such as age and tumor stage, etc.

Reply: We have included new data demonstrating the association between the expression of the miR-183 family and confounders using a binary logistic regression model in our revised manuscript. Results showed that lung cancer patients over 60 years old or with squamous cell lung carcinoma had higher tumor miR-96 expression levels. Patients with squamous cell lung carcinoma or tumor invasive to lung membrane had higher levels of tumor miR-183. Patients with poor differentiation had higher levels of miR-96, miR-182 and miR-183 in sera (see page 12 and 28 in Table 2). In addition, we also examined if the expressions of miR-96, miR-182, and miR-183 in tumors and sera were correlated with clinical-pathological features of the patients. With hazard ratios (adjusted for sex, age and tumor stage) of 9.637, 7.163, 8.616, $P = 0.005$, 0.010, and 0.005 respectively in tumors and with hazard ratios (adjusted for sex, age and tumor stage) of 5.512, 5.327, 5.972 yielded $P = 0.027$, 0.030, and 0.022 respectively (Table 3) in sera. This suggested that miR-96, miR-182 and miR-183 in tumor and sera independently contributed to the overall survival of lung cancer patients. We have included the above in the revised manuscript (see page 13 in the second paragraph).
Minor Essential Revisions
3) The manuscript contains typographical errors, fragments, awkward phrasing and improper use of punctuation and should be thoroughly reviewed for English grammar.
   Reply: We have corrected these errors.

Discretionary Revisions
4) The conclusion that miR-183 is involved in tumor metastasis due to its overexpression in SCLC compared to NSCLC is an overstatement, as these are distinct diseases and the former does not represent a progression of the latter. Lacking additional experiments to support the validity of the statement, the authors should rephrase.
   Reply: We have revised these sentences in our revised manuscript (see page 11 on the third paragraph and page 15 on the second paragraph).

Reviewer: Lynne Bemis
Minor essential Revisions
1) The authors should also give more details concerning how much RNA was utilized in the reverse transcription assays and several of the conclusions are seeming without experimental support (see below)
   Reply: We have included more detailed information in our revised manuscript (see page 9 on the paragraph 1).
2) The following sentence in the abstract does not make sense.
   To identify specific miRNAs with diagnostic and prognostic value for patients with lung cancer, and reveal any correlation between their expression profiles and patient survival.
   Reply: We have revised this sentence in the abstract in our revised manuscript (see page 3)
3) There does not seem to be any experimental explanation for the authors to draw this conclusion about metastasis.
   These results imply that the miR-183 family of miRNAs might participate in the process of cancer metastasis.
   Reply: Appropriate revisions have been made in the revised manuscript (see page 15 on the paragraph 2).
4) In this next section we need a more detailed explanation of why the authors conclude that this family of microRNAs is involved in progression.
   Their statement: This suggests that upregulation of the miR-183 family might contribute to lung cancer progression. Needs to be explained in more detail because it is hard to determine how they come to this conclusion with the current study.
   Reply: The reviewer is right. Because of the preliminary limited data, we have removed this sentence in our revised manuscript (see page 15 on the paragraph 2).
4) A more comprehensive discussion of about the previous studies of the miR-183 Family could be included and it ends abruptly with the statement: This needs to be determined. Reply: More discussion has been included (see page 15 on the paragraph 3).

5) More detailed methods would be helpful, for example what was the purpose of the 15% polyacrylamide gels?
Referring to: and run on a denaturing 15% polyacrylamide gel.
Reply: We have corrected this error in our revised manuscript (see page 8 on the paragraph 1).

6) Several small errors are noted:
Transcription followed by real-time quantitative
The results showed that high expressions of
The sentence should read lung cancer primary tissues because lung primary tissues can be misconstrued as normal lung.
"Family were highly expressed in lung primary tissues and sera"
Reply: We have corrected these errors in our revised manuscript (see below).
“Transcription followed by real-time quantitative” was corrected by “quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR)” (see page 3 on the second paragraph and page 5 on the second paragraph).
“The results showed that high expressions of” was corrected by “Results showed that high expression of” (see page 13 on the first paragraph).
“Lung primary tissues” was corrected by “lung cancer primary tissues” (see page 15 on the second paragraph).