Author's response to reviews

Title: MicroRNA Expression Profile in Exosome Discriminates Extremely Severe Infections from Mild Infections for Hand, Foot and Mouth Disease

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Author's response to reviews: see over
Aug 28, 2014

Dear Editors,

I, along with my co-authors, would like to ask you to consider the attached manuscript entitled ‘MicroRNA Expression Profile in Exosome Discriminates Extremely Severe Infections from Mild Infections for Hand, Foot and Mouth Disease’ for publication in the BMC Infectious Diseases as an research article.

In this study, we reveal the miRNA profiling of exosomes in extremely severe hand, foot, and mouth disease (ESHFMD) and mild hand, foot, and mouth disease (MHFMD) to improve the HFMD diagnosis. The results showed that miR-671-5p, miR-16-5p, and miR-150-3p provided higher diagnostic accuracy for ESHFMD. They may relate to neurotrophin signalling pathway and considered to have clinical value in diagnosing of ESHFMD.

We believe that the findings of this study are relevant to the scope of your journal and will be of interest to its readership.

This manuscript has not been published or presented elsewhere in part or in entirety, and is not under consideration by another journal. All the authors fulfill the criteria for authorship that have been specified by the journal. All study participants provided informed consent, and the study design was approved by the appropriate ethics review boards. All the authors have approved the manuscript and agree with submission to your esteemed journal. There are no conflicts of interest to declare.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,
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Reviewer's report
Title:MiR-671-5p, miR-16-5p and miR-150-3p in Exosomes are Potential Biomarkers for Extremely Severe Hand, Foot, and Mouth Disease
Version:3 Date:4 May 2014
Reviewer:Dong-Yan Jin
Reviewer's report:
In this paper Jia et al. reported a miRNA profiling study with exosomes extracted from sera of a very small cohort of patients with mild or extremely severe HFMD. The work is original and might be of some interest. However, the sample size is too small and the results do not support the conclusions made. As it is, the paper is not ready for publication in any journal.
1) The sample is too small and the etiology of HFMD is not established or reported.
We increased the sample size and supplemented 10 groups (normal, MHFMD, ESHFMD) experimental data in the revised manuscript.

2) The differentially detected miRNAs are also found in other conditions such as critical limb ischemia (Circ Res 112:335-346, 2013) and colon cancer (PLoS One 8:e84256, 2013). They can unlikely serve as biomarkers for severe HFMD.

We should collect the clinical samples such as serum from critical limb ischemia and colon cancer patients to verify the specificity of these miRNAs that we screened from HFMD. But these clinical samples are difficult to collect, and we can not performed the related experiments. So we modified the title and content of the manuscript.

3) Exosomes should be purified and the procedures for exosome isolation and purification should be detailed.

We have attached the relevant literature for exosome extraction kit in the revised manuscript.

4) The quality of exosomes is in doubt. Additional proteins such as CD9, Hsp90alpha/beta and flotillin should be examined in the validation experiments.

We supplemented the validation experiments of CD9, Hsp90alpha/beta and flotillin as biomarkers of exosome.

5) RT-qPCR validation should be performed with an independent cohort of more patients.

We increased the sample size and supplemented 10 groups (normal, MHFMD, ESHFMD) experimental data for RT-qPCR validation.

6) Pooled samples were used in protein validation. This is too crude and the results are unreliable. Many additional assays have to be carried out to verify the predicted targets. This part is not highly relevant to the key message of the paper and should therefore be deleted.

We agreed with the reviewer’s comment and deleted the content of proteomics.

**Reviewer's report**

**Title:** MiR-671-5p, miR-16-5p and miR-150-3p in Exosomes are Potential Biomarkers for Extremely Severe Hand, Foot, and Mouth Disease

**Version:** 3

**Date:** 19 May 2014

**Reviewer:** Hanzhong Wang

**Reviewer's report:**

This interesting study provides evidence for microRNAs in the exosome as potential biomarkers for extremely severe hand, foot, and mouth disease. The three miRNAs (miR-671-5p, miR-16-5p and miR-150-3p) significantly differentially expressed in MHFMD and ESHFMD serum samples compared to healthy children. And the authors conclude that these miRNAs may have clinical value in differentially diagnosing and can be used in rapid diagnosis or therapy for ESHFMD.

**Major comments:**

1. As mention in the paper, the isolation of exosomes needs ultracentrifugation and special kits, and the identification needs TEM and western blotting. These are not so convenient and easy to operate. So it is not practical for rapid diagnosis or therapy for ESHFMD.

We are very grateful to the reviewer’s comment. The traditional method is to extract exosome by ultracentrifugation, but in recent years several companies produced exosome
extraction kits to rapidly extract exosomes, and we have attached the relevant literature for exosome extraction kit in the revised manuscript.

2. The exosomes contain proteins, miRNAs, and mRNAs, and these molecular can be transferred by exosomes to other cells. So the microRNAs in the exosomes may target proteins in the whole tissue and cells but not only in the exosomes. Thus, The pooled proteins for 2-DE analysis to identified differential expressed proteins from ESHFMD, MHFMD patient should be the whole tissue or cell protein.

The manuscript focused on miRNAs from exosomes, The proteins of exosomes is not highly relevant to the key message of the paper. So we decided to delete the content of proteomics after discussion.

3. It seems that the sixth paragraph in the Discussion (line 1-6, page 24) is related to the conclusion and hypothesis. It is suggested to delete this paragraph.

We agreed with the reviewer’s comment and deleted the paragraph.