

Format for ANSWERING REVIEWERS

August 11, 2014



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11719-review.doc)

Title: MicroRNA profiling of the intestine during hypothermia circulatory arrest in swine

Author: Weibin Lin, Guangxian Chen, Mengya Liang, Xiao Yang, Han Qin, Jianping Yao ,Kangni Feng, Zhongkai Wu*

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 11719

The manuscript has been improved according to the suggestions of the reviewers:

1. Format has been updated
2. Revisions have been made according to the suggestions of the reviewers

Reviewer (1) [Reviewer No. 02890068]

Comment : Which statistical program used name it please

Response :

The statistical program named SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used, and this information has been added to the Material and Methods section.

Reviewer (2) [Reviewer No. 02397853]

Comment :

The paper by Lin et al. addressed that the difference in microRNAs expression levels during HCA, which involves in the specific miRNAs on the process of HCA. The experimental plans are organized.

Response :

We are grateful to the reviewer for his/her appreciation regarding the quality of our work.

Reviewer (3) [Reviewer No. 02928799]

Comment :

This manuscript concerns HCA-regulated miRNA expression in swine intestine. By conducting a miRNA array experiment, the authors targeted some miRNAs which might be important and significant in the HCA or I/R-induced tissue injury. 1. Overall, the research design is very decent with adequate animal experiment, miRNA sample preparation, and array profiling. 2. The authors should provide more evidence to proof the results from the array data. Though GeneChip miRNA Array from Exiqon is well known for high accuracy, the authors should do at least one more validation specifically for those miRNAs with significant changes listed in the Table 1. 3. Alternatively, the authors should provide data on the expression levels of the candidate genes regulated by those

significantly changed miRNAs. This would help to proof the significance of these miRNAs as well as to link the miRNAs and pathological observation shown in Figure 1a. 4. The English writing in this manuscript requires a modification by a native English speaker. The name who performed the first HCA experiment is Bigelow, not Biglow (Introduction, line 1).

Response :

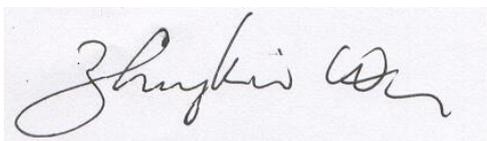
The observant reviewer has raised excellent points. 1. The technique mentioned in our method is a realtime PCR miRNA array, which is different from a GeneChip miRNA array. The realtime PCR technique is a common technique used to confirm miRNA array data. This technique was utilized by other studies without the need for validation such as "Identification of serum microRNA profiles in colon cancer" (PMID 23558896) and "Plasma miRNAs as Biomarkers to Identify Patients with Castration-Resistant Metastatic Prostate Cancer" (PMID 23574937). As for our expression in manuscript may confuse the reader, our expression data were revised for clarification and the modifications are highlighted in our revised manuscript. 2. Many candidate genes are potentially targeted by the 7 identified miRNAs; therefore, we assessed the mutual target genes of these miRNAs. Only a few of these target genes were validated in other studies, suggesting that the direct relationship between the gene and pathological observation shown in our Figures is difficult to identify. Our research team is currently performing the further study suggested by the esteemed reviewer; however, the results we got is not ideal to find out the connection between miRNA and gut barrier. We are still aiming to understand the relationship between the histological findings and the identified miRNAs. According to the reviewer's valuable comments, we have added the absence of this experiment as a limitation in our revised manuscript. We hope the initial data of our study may inspire other researchers to determine the connection between these miRNAs and the gut barrier. 3. The English writing in this manuscript was modified by American Journal Experts. The name of the person who performed the first HCA experiment was corrected as "Bigelow".

3 References and typesetting were corrected

The PMID and DOI were added in the revised manuscript, except for the reference "Croome KP, Kiaii B, Fox S, Quantz M, McKenzie N, Novick RJ. Comparison of gastrointestinal complications in on-pump versus off-pump coronary artery bypasses grafting. *Can J Surg* 2009 2009-04-01; 52(2): 125-128". However, the free full text is available for readers online immediately by searching its PMID.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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