August 30, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3857-Edited.doc).

Title: Overexpressed microRNA-155 dysregulates intestinal epithelial apical junctional complex in experimental severe acute pancreatitis

Author: Rui Tian, Ruilan Wang, Hui Xie, Wei Jin, Kanglong Yu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3857

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

2 Revision has been made according to the suggestions of the reviewer

- Reviewer 1 (Grade C, major revision)

Rui Tian and co-workers present a study examining how MicroRNA-155 (miR-155) regulates epithelial apical junctional complex in severe acute pancreatitis. The study was carried out in a caerulein/LPS-induced mouse model of acute pancreatitis. Samples were collected 3 hours after the last injection. The authors demonstrated evidence of severe acute pancreatitis. miR-155 expression increased compared to controls and was associated with a decrease in ZO-1, E-cadherin, and RhoA expression. The authors conclude that these results suggest that severe acute pancreatitis decreases intestinal barrier function by decreasing synthesis key junctional complex proteins via mi-R-155 signaling.

Response: Thank you for your kind review.

Critique 1. The question that arises is whether the findings are specific for severe acute pancreatitis? The model uses two substances to induce severe pancreatitis. Does either of these substances directly induce the findings in intestine? Did you perform controls in which cerulean alone and LPS alone are given without induction of pancreatitis? Is there evidence that pancreatitis caused by other mechanisms result in these changes?

Response: As mentioned in our manuscript, apical junctional complex (AJC) is the major structure maintaining intestinal barrier function. Any factors that damage AJC will impair intestinal barrier function. Our results showed that excessive inflammatory response occurred in severe acute pancreatitis (SAP) and manifested as a cascade-like release of

proinflammatory factors, such as TNF-α. Increased serum TNF-α induced miR-155 overexpression in SAP intestinal epithelia, and subsequently destructed AJC by acting on RhoA-ZO-1/E-cadherin signaling pathway. It is possible that increased TNF-α release in other inflammatory diseases will also destruct AJC through the aforementioned pathway. It is known that SAP features of excessive inflammatory response at the early stage, and TNF-α is believed to be the first emerging and one of the key proinflammatory factors implicated in systemic inflammatory response syndrome. Therefore, it is more likely that TNF-α-miR-155-RhoA-ZO-1/E-cadherin is the major signaling pathway contributing to intestinal barrier dysfunction in SAP.

Sequential intraperitoneal injection of caerulein and LPS is the most common method to induce experimental SAP in mouse model. The experimental protocol including injection dose has been well established in current literature. It has been reported that injection of caerulein alone or LPS alone cannot result in any pancreatic pathology mimicking SAP in humans. It remains unknown whether injection of caerulein alone or LPS alone can destruct intestinal barrier. In our study, macroscopy and microscopy showed no marked pathology, such as edema, erosion, or ulceration, on intestinal serosal side in SAP mice, while these pathologies were located on the mucosal side. Therefore, intestinal AJC destruction in SAP mice resulted from secondary systemic inflammatory response rather than directly intraperitoneal injection of caerulein and LPS. It is advisable to use mice injected with caerulein alone and LPS alone as controls.

As aforementioned, any factors inducing systemic inflammatory response may also contribute to the occurrence of SAP. In our previous work, we injected sodium taurocholate hydrate to the pancreatic and bile ducts to induce experimental SAP in rats, in which excessive inflammatory response, such as marked serum TNF-α increase, emerged.

2. It is difficult to understand how the miR-155 target genes were predicted using miRTarBase, RNA22, and PicTar. This explanation needs to be expanded.

Response: MicroRNA binds to its target genes via specific binding sites in the 3′ UTR. This specific structural pairing can be used to predict the target genes for miR-155. The miRTarBase database (http://mirtarbase.mbc.nctu.edu.tw)[?] is a database curating experimentally validated microRNA-target interactions; RNA22 (http://cbcsrv.watson.ibm.com/rna22.html)[?] and PicTar (http://pictar.mdc-berlin.de)[?] are

two computational methods used to analyze the target genes for a given gene. Briefly, on the miRTarBase database search page enter 'mouse' in the box of 'species' and 'miR-155' in the box of 'microRNA'; the database will automatically list all possible target genes for miR-155, and RhoA gene (ID: MIRT000949) is one of them.

3. The intestine when examined showed necrosis of cells and shedding of mucosal cells in the intestine of the pancreatitis mice. How does this loss of cells translate in measurement of the proteins in question? Was it down regulated or lost during intestinal damage?

Response: Loss of intestinal epithelial cells was observed in SAP mice. All Western blotting assays of RhoA, ZO-1, and E-cadherin were performed with β-actin as an internal control; the expression levels of target proteins were normalized to that of the internal control, which excluded the confounding effect of epithelial cell loss in SAP mice. Therefore, it could be concluded that the expressions of these target proteins were downregulated in SAP intestinal epithelia.

4. Minor: The manuscript is easy to understand, but the abstract has abbreviations that are not defined. For example, the abbreviated term for MicroRNA-155 should be placed in parenthesis after it is first mentioned. Also, it would be best to define the measured proteins in the abstract as those important for intestinal barrier function. The manuscript and study is straight forward and, except for the prediction of target genes, easy to follow. Like all pancreatitis models, there is question about the correlation of the findings in the specific pancreatitis model with pancreatitis in humans. In this case, one must determine if the effects studied in the mice were due to severe acute pancreatitis or the agents used to induce it. The findings are interesting, but what is the significance of these findings to the prevention or treatment of pancreatitis?

Response: Thank you for your kind review again. All the abbreviations used in the Abstract have been spelled in the full form. Also the biological roles of target proteins, such as RhoA, ZO-1, and E-cadherin, have been defined in the Abstract. The method of miR-155 target gene prediction has been described in more detail.

It is true that no single technique can establish an experimental SAP model identical to human SAP. Sequential intraperitoneal injection of caerulein and LPS is the most common method to induce experimental SAP in mouse model. The absence of serosal pathology in SAP mice could probably exclude the direct effects of injected agents on mice.

Anti-TNF-α therapy has been experimented in the treatment of SAP. Our preclinical study suggested a possible modality of choice for treating SAP or preventing SAP disease progression by targeting at miR-155 overexpression and RhoA underexpression to restore damaged AJC and intestinal barrier in SAP.

• Reviewer 2 (Grade A, accept)

To the authors: Congratulations for the excellent work-liberatocaboclo@gmail.com

Response: Thank you for your review.

• Reviewer 3 (Grade A, minor revision)

ESPS Manuscript NO: 3857 MicroRNA-155 regulates intestinal epithelial apical junctional complex in severe acute pancreatitis It is well designed study with new interesting findings. There are few typing errors. The close correlation between intestinal barrier dysfunction and poor prognosis of SAP is well known.

The authors reported interesting points 'for the first time': 1) The contribution of miR-155 in intestinal barrier dysfunction in SAP and the participation of TNF-α in the early inflammatory responses in SAP with AJC structure damage via miR-155 pathway. 2) miR-155 acts on the target gene of RhoA and inhibits protein synthesis of ZO-1 and E-cadherin, which are the major components for TJs and AJs, respectively. 3) TNF-α-miR-155-RhoA pathway may contribute on to a better understanding of etiology and the mechanisms of intestinal barrier dysfunction in SAP.

Response: Thank you for your review.

3 References and typesetting were corrected

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

Sincerely yours,

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