ANSWERING REVIEWERS

December 5, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6815-review.doc).

Title: MicroRNAs: New therapeutic targets for intestinal barrier dysfunction

Author: Lin Zhang, Jian Cheng, Xiaoming Fan

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6815

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

For reviewer 02109990

(1) The case of multiple myeloma and its possible treatment through the use of miRNA therapeutics should be discussed citing the following Emerging pathways as individualized therapeutic target of multiple myeloma: Misso G et al. Expert Opin Biol Ther. 2013 Jun;13 Suppl 1:S95-109. doi: 10.1517/14712598.2013.807338

A: Thank you for your constructive advice. The case of multiple myeloma and its possible treatment through the use of miRNA therapeutics were discussed in the last paragraph and the related paper named" Emerging pathways as individualized therapeutic target of multiple myeloma" was cited in the manuscript.

(2) The authors should cite the involvement of miR29b in osteoclastic cell differentiation (Rossi M et al. J Cell Physiol. 2013 Jul;228(7):1506-15) and epigenetic regulation of cell cycle (Amodio N et al. Cell Death Dis. 2012 Nov 29;3:e436 and Amodio N et al. Oncotarget. 2012 Oct;3(10):1246-58.).

A: Thank you very much. The involvement of miR-29 in multiple myeloma was discussed in the last paragraph and the related papers were cited.

(3) The addition of a figure depicting miRNA involvement in the pathogenesis and diagnosis of IBD could be helpful for the reader.

A:We appreciate your advice very much. A figure depicting miRNA involvement in IBD was added in the third paragraph. Please see the figure named Figure 1. In this figure, we summarized the role of microRNAs in IBD.

For review 00157873

General comment: in the review "MicroRNAs: new therapeutic targets for treatment of intestinal barrier dysfunction" the Authors (Lin Zhang, Jian Cheng, Xiao-Ming Fan) try to give to the reader a picture of what miRNAs are, their implication in inflammatory processes and their possible role as therapeutic targets. Results from the specific literature are reported in small chapters, which render easier to the reader to get in touch with this interesting and complex topic. In the present form, however, the text results somehow redundant and repetitive; see for example the following identical

paragraphs reported on different pages: Pag.6: Furthermore, NOD2, an intracellular bacterial sensor of the Nod-like receptor (NLR) family, most expressed in macrophage, can sense the presence of MDP, a component of the peptidoglycan cell wall from both Gram-positive and Gram-negative bacteria[62]. NOD2 activation results in pro-inflammatory and anti-bacterial molecule production dependent on cell signaling pathways mediated by RICK/RIP2, NF-κB and MAPKs. NOD2 mutations have been identified in the CD[63]. Most recently, Ghorpade et al found that miR-146a mediated NOD2-SHH signaling can regulates the gut inflammatory in a mouse model of IBD[64] And pag 8-9; Furthermore, NOD2, an intracellular bacterial sensor of the Nod-like receptor (NLR) family, can sense the presence of MDP, a component of the peptidoglycan cell wall from both Gram-positive and Gram-negative bacteria. NOD2 activation results in pro-inflammatory and anti-bacterial molecule production dependent on cell signaling pathways mediated by RICK/RIP2, NF-κB and MAPKs. Most recently, Ghorpade et al found that miR-146a mediated NOD2-SHH signaling can regulates the gut inflammatory in a mouse model of IBD. On the contrary, the final paragraph dealing with the role of miRNAs as biomarkers and new therapeutic targets is very short and should be expanded. The paper results difficult to read because of the numerous language mistakes; a deep revision of the text-language is needed. Minor comments: Reference list appears complete but for the barrier function in health and disease (In the Introduction, N°1-5); the authors deal with this aspect in a sketchy way, even considering the required shortness; this should be changed or enriched with more recent papers citations. Throughout the text: "miRNA" and "small non coding RNA" etc should be mentioned as "miRNAs", "small non coding RNAs" etc when appropriate and the related verbal forms should be corrected.

A: Thank you very much for your favorable evaluation.

The redundant and repetitive text in the chapter of "miRNAs and inflammation" was revised;

Deep revision of the text language was done by the professional company;

The paragraph in the introduction was revised as follows:

Tight junctions and its associated proteins, including claudins, occludin, and zonulaoccludens, and so on, are theapical-most adhesive junctional complexes and act as a structural and functional barrier against paracellular permeation of luminal substances[2-4]. A breakdown or disruption of the epithelial barrier has been implicated as an essential determinant in the predisposition to intestinal inflammation and a number of inflammatory disorders, such as Crohn's disease[5], ulcerative colitis[6, 7], celiac disease[8], and a series of infectious diarrheal syndromes[9, 10].

And Most recent references related with the introduction were cited, they were

2 **Groschwitz KR**, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009; **124**: 3-20; quiz 21-22 [PMID: 19560575 DOI: 10.1016/j.jaci.2009.05.038]

3 **Groschwitz KR**, Ahrens R, Osterfeld H, Gurish MF, Han X, Abrink M, Finkelman FD, Pejler G, Hogan SP. Mast cells regulate homeostatic intestinal epithelial migration and barrier function by a chymase/Mcpt4-dependent mechanism. *Proc Natl Acad Sci U S A* 2009; **106**: 22381-22386 [PMID: 20018751 PMCID: 2799737 DOI: 10.1073/pnas.0906372106]

4 **Ulluwishewa D**, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutrition* 2011; **141**: 769-776 [PMID: 21430248 DOI: 10.3945/jn.110.135657]

5 **Sartor RB**. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 390-407 [PMID: 16819502 DOI: 10.1038/ncpgasthep0528]

6 **Clavel T**, Haller D. Bacteria- and host-derived mechanisms to control intestinal epithelial cell homeostasis: implications for chronic inflammation. *Inflamm Bowel Dis* 2007; **13**: 1153-1164 [PMID:

17476679 DOI: 10.1002/ibd.20174]

7 **Werner T**, Haller D. Intestinal epithelial cell signalling and chronic inflammation: From the proteome to specific molecular mechanisms. *Mutation Res* 2007; **622**: 42-57 [PMID: 17628614 DOI: 10.1016/j.mrfmmm.2007.05.010]

8 **Brandtzaeg P**. Update on mucosal immunoglobulin A in gastrointestinal disease. *Curr Opin Gastroenterol* 2010; **26**: 554-563 [PMID: 20693891 DOI: 10.1097/MOG.0b013e32833dccf8]

9 **Gecse K**, Roka R, Ferrier L, Leveque M, Eutamene H, Cartier C, Ait-Belgnaoui A, Rosztoczy A, Izbeki F, Fioramonti J, Wittmann T, Bueno L. Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. *Gut* 2008; **57**: 591-599 [PMID: 18194983 DOI: 10.1136/gut.2007.140210]

10**Gadewar S**, Fasano A. Current concepts in the evaluation, diagnosis and management of acute infectious diarrhea. *Curr Opin Pharmacol* 2005; **5**: 559-565 [PMID: 16207535 DOI: 10.1016/j.coph.2005.08.002]

The "miRNA" and "small non coding RNA" etc were correct with "miRNAs", "small non coding RNAs" etc when appropriate. And indicated with yellow color highlight in the text.

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3 Recent references were added and the total references were more than 100.

2 **Groschwitz KR**, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009; **124**: 3-20; quiz 21-22 [PMID: 19560575 DOI: 10.1016/j.jaci.2009.05.038]

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36 **Zabolotneva AA**, Zhavoronkov AA, Shegay PV, Gaifullin NM, Alekseev BY, Roumiantsev SA, Garazha AV, Kovalchuk O, Aravin A, Buzdin AA. A systematic experimental evaluation of microRNA markers of human bladder cancer. *Front Genet* 2013; **4**: 247 [PMID: 24298280 DOI: 10.3389/fgene.2013.00247]

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39 **Sun F**, Chen HG, Li W, Yang X, Wang X, Jiang R, Guo Z, Chen H, Huang J, Borowsky AD, Qiu Y. Androgen receptor splice variant AR3 promotes prostate cancer via modulating expression of autocrine/paracrine factors. J Biol Chem 2013 [Epub ahead of print] [PMID: 24297183 DOI: 10.1074/jbc.M113.492140]
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96 **Ghorpade DS**, Sinha AY, Holla S, Singh V, Balaji KN. NOD2-nitric oxide-responsive microRNA-146a activates sonic hedgehog signaling to orchestrate inflammatory responses in murine model of inflammatory bowel disease. *J Biol Chem* 2013[Epub ahead of print] [PMID: 24092752 DOI: 10.1074/jbc.M113.492496]

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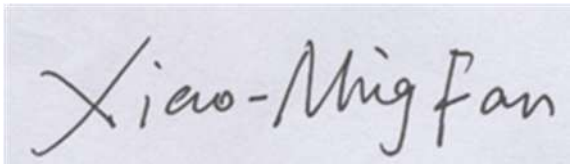
Sincerely yours,



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