Format for ANSWERING REVIEWERS

August 1, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2429-review.doc).

Title: Interleukin-22 ameliorates liver fibrogenesis by attenuating HSC activation and downregulating the levels of inflammatory cytokines

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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
(1) Reviewer #1:
Question: (1) The authors state in the IL-22 could attenuate HSC activation yet there is only circumstantial evidence presented in the manuscript regarding this point. Do HSCs express IL-22R? (2) Figure 1 could be moved to supplemental material. (3) Numerous typographic and syntax errors throughout the manuscript that need extensive proofreading.

Answer: Thank you for the important comment. (1) HSCs do express IL-22R mainly based on following reports (①Feng Det al (2012) Interleukin-22 promotes proliferation of liver stem/progenitor cells in mice and patients with chronic hepatitis B virus infection. Gastroenterology 2012; 143:188–98 e7. ②Kong X et al (2012) Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis. Hepatology 2012; 56: 1150–1159). In our following study, we would detect the levels of IL-10R2 and IL-22R1 expression on HSCs, and comfirm IL-22 attenuate HSC activation by IHC and WB. (2) Fibrogenesis is a dynamic process which is important to our liver study. Thus, to dynamically evaluate fibrogenesis, we have conducted in vivo experiments with different time point (Figure 1): Liver fibrosis was assessed by histology and Masson's trichrome staining. Alpha smooth muscle actin (α-SMA) expression was determined by immunohistochemical staining. (3) Typesetting and grammar errors have been proofreading.
(2)Reviewer #2:
Question: (1) In the introduction, the authors stated that IL-22 protects against T cell hepatitis. The first reference that demonstrated this effect should be cited. (Radaeva et al. IL-22 plays a protective role in T cell hepatitis: IL-22 is a survival factor for hepatocytes via activation of STAT3. Hepatology 2004, 39:1332-1342). (2) In the method, the authors described "the mice were given 300ug/kg of rmIL-22(1ug/g; R&D Systems". 300 ug/kg is not equal to 1ug/g" (3) In Fig. 2A, it shows gated CD4+ T cells with IL-22 and IL-17 staining. Only IL-22 staining was detected. This seems not correct. (4) The authors stated Th22 and Th17 cells. Th17 cells are known to produce IL-22. Did the authors detected Th17 cells that produce both IL-17 and IL-22? (5) Fig. 6 shows that IL-22 treatment reduces Th22, Th17, and Th1 cells. What are underlying mechanisms?

Answer: Thank you for your important issue. (1) The first reference demonstrated that IL-22 protects against T cell hepatitis has been cited. (Radaeva et al. IL-22 plays a protective role in T cell hepatitis: IL-22 is a survival factor for hepatocytes via activation of STAT3. Hepatology 2004, 39:1332-1342). (2) In the method, "300 ug/kg shoud be equal to 0.3ug/g instead of 1ug/g", this mistake has been corrected. (3) Actually in the study, we have dyed the IL - 22, IL - 17 andIFN-γ at the same time. However, IL - 17 dyeing effect is not good with APC fluorescence (figure 2A). In order to better demonstrate IL-17 effect, IL-17 staining was detected by PE dyeing under the same experimental mice (figure 2B). (4) In this article, we evaluated the total levels of Th22 cells, Th17 cells and IL-22, IL-17A in different group. Moreover, we confirmed frequencies of Th22 and Th17 cells were elevated in hepatic fibrosis. In our following experiment, we would consider to detect Th17 cells that produce both IL-17 and IL-22. (5)IL-22 treatment reduces Th22, Th17, and Th1 cells. The underlying mechanisms are that IL-22 inhibits Th22, Th17 and Th1 responses likely through modulating the release of the cytokines (TNF-α, IL-6 and IL-1β).

(3)Reviewer #3:

Question: 1-In abstract, Introduction :MaleBALB/C....etc, needs language corrections. 2-In discussion:Shiz etal,indicated that exhibit....etc,needs language corrections. 3-You have mentioned in discussion that the simultaneous rise of Th22 and IL22,suggest that IL22 Is secreted by Th22 cells,although you have conducted on your research on this fact that you have presented in introduction,so,I think this statements has to be revised.The same applies for what mentioned for Th17 and Th1.

Answer: Thank you for your suggestion. (1, 2) In abstract and discussion, we have corrected language. (3) These statements which you have mentioned have been revised.

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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