Dear editor Ma,

Thank you for your consideration of our manuscript, *Yiguanjian decoction enhances fetal liver stem/progenitor cell-mediated repair of liver cirrhosis through regulation of macrophage activation state*. We have made corrections to the paper per the reviewers' suggestions. Point-by-point responses to comments are as follows:

Reviewer #1:

Title: Yiguanjian decoction enhances fetal liver stem/progenitor cell-mediated repair of liver cirrhosis through regulation of macrophage activation state. The authors aimed to investigate if Yiguanjian Decoction (YGJ) possesses beneficial effects on CCl4-induced fibrosis in rats and to assess if YGJ combination with fetal liver stem/progenitor cell (FLSPC) increases the antifibrotic activity.

The authors found that FLSPC transplantation improved liver function and histopathology and inhibited the activation of the non-canonical Wnt signaling pathway, while activating the canonical Wnt signaling pathway. In addition, the results indicate that YGJ enhanced the therapeutic effects of FLSPCs by inhibiting the canonical and enhancing the non-canonical Wnt signaling pathways. They concluded that YGC in combination with FLSPC may be useful for the treatment of human fibrosis.

Comments:

1. The authors utilized a model of CCl4 plus 2-AAF to induce cirrhosis. Why? Please justify the model. Why not CCl4 alone?

Response. Hepatic parenchymal cells (hepatocytes) have a strong ability to regenerate. Mature hepatocyte proliferation, a self-protective response to liver injury, is the primary defense mechanism by which the body maintains its

homeostasis. In order to observe the role of stem/progenitor cells in liver injury, it is often necessary to inhibit the regeneration of hepatocytes. In the field of research on liver regeneration, a large number of studies have used 2-AAF to establish animal models in which hepatocyte proliferation was suppressed, and the hepatic stem cells and/or hepatic progenitor cells are activated [1-4]. In present study, in order to observe the therapeutic effect of fetal liver stem/progenitor cells, we used 2-AAF/CCl4 rat model, a well-characterized model with a long history in investigations of stem-cell-mediated liver regeneration, where proliferation of parenchymal cells is impaired and hepatic stem cells are activated, resulting in hepatic progenitor/oval cell proliferation.

References:

[1] Lorenzini S, Bird TG, Boulter L, Bellamy C, Samuel K, Aucott R, Clayton E, Andreone P, Bernardi M, Golding M, Alison MR, Iredale JP, Forbes SJ. Characterisation of a stereotypical cellular and extracellular adult liver progenitor cell niche in rodents and diseased human liver. Gut, 2010, 59(5):645–654.

[2] Thorgeirsson SS. Hepatic stem cells in liver regeneration. FASEB J. 1996, 10(11):1249-1156.

[3] Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology, 2004, 39(6): 1477–1487.

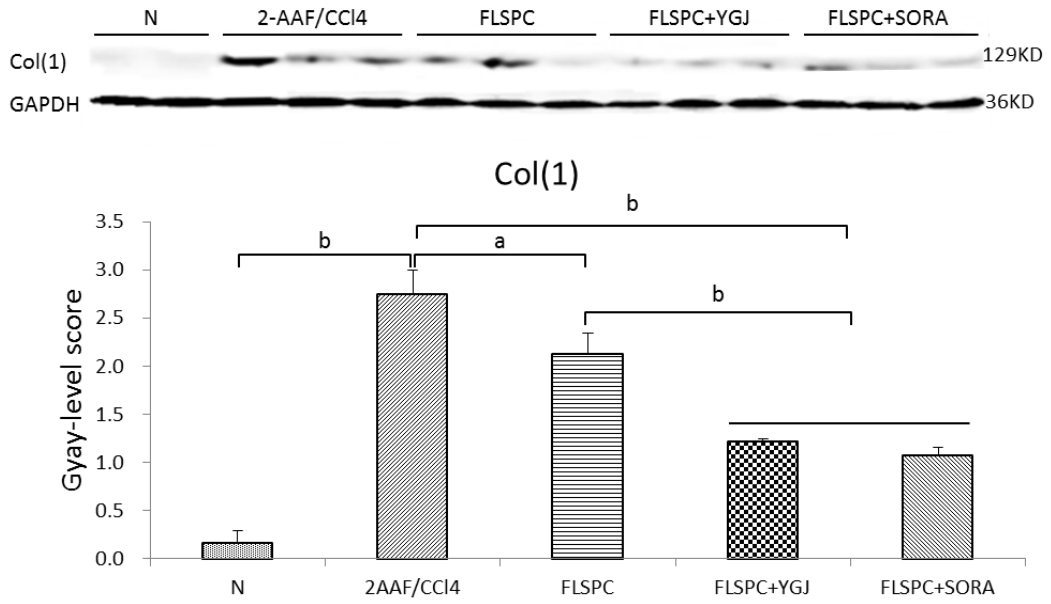
[4] Wang J, Koyota S, Zhou X, Ueno Y, Ma L, Kawagoe M, Koizumi Y, Okamoto H, Sugiyama T. Expression and localization of regenerating gene I in a rat liver regeneration model. Biochem Biophys Res Commun. 2009, 380(3):472-477.

2. In addition to serious red, another quantitative marker of fibrosis, like hydroxyproline or IHQ o WB of collagen is recommended for in vivo studies.

Response. In this study, we used the Leica Scan 400 to perform a complete scan of the tissue of each section. We then used the analysis software to calculate the positive area of the serious red in the whole section. In this way, it should be able to objectively indicate the content of collagen in liver tissue.

In addition, we also examined the protein expression of collagen type 1 (Col(1)) and the results were consistent with the positive area of serious red. The data are shown in Supplementary Figure 3.

Supplementary Fig. 3 The protein expression of Col(1)



Supplementary Figure 3. The protein expression of Col(1). (a) Immunoblotting for Col(1). (b) The gray-level score indicates the immunoblotting histogram for Col(1). [a] $P < 0.05$; [b] $P < 0.01$. N, normal control group; 2-AAF/CCl4, 2-acetylaminofluorene/carbon tetrachloride group; FLSPC, fetal liver stem/progenitor cell group. FLSPC+YGJ, FLSPCs plus Yiguanjian decoction group; FLSPC+SORA, FLSPCs plus sorafenib group.

3. Does the NF-kappaB proinflammatory factor plays a role? Why it was not measured?

Response. NF-κB is a recognized proinflammatory factor, and activation of NF-κB signaling pathway can promote the progression of inflammatory response and liver fibrosis. In this way, to inhibit NF-κB activation and

decrease the secretion of pro-inflammatory cytokine such as TNF-α and IL-6 becomes one of the hot spots of anti-fibrosis research.

In this work, we focused on the relationship between Yiguanjian Decoction (YGJ), fetal liver stem/progenitor cell differentiation, and macrophage activation status. Although it is clear that YGJ has a regulatory effect on macrophage activation status, the molecular mechanism by which it exerts that effect is still unclear. At present, we are studying the molecular mechanism by which YGJ regulates macrophage polarization, and we will observe the effect of YGJ on NF-κB signaling pathway. Thank you for your suggestion.

4. Professional edition must be performed again, the manuscript is very bad written. I am surprised that the authors uploaded a certi¬ficate of English language editing by LetPub.

Response. Thank you for your valuable comments. The manuscript has been closely examined by LetPub.

Reviewer #2: References to be updated.