Dr. Jin-Lei Wang
Director, Editorial Office
Baishideng Publishing Group Co., Limited

Dear Dr. Jin-Lei Wang,

Pleas find enclosed the edited and revised manuscript in PDF format (file name: 27269-Revised manuscript).

Title: DNA methylation of angiotensin II receptor gene in nonalcoholic steatohepatitis-related liver fibrosis

Author: Kiyoshi Asada, Yosuke Aihara, Hiroaki Takaya, Ryuichi Noguchi, Tadashi Namisaki, Kei Moriya, Masakazu Uejima, Mitsuteru Kitade, Tsuyoshi Mashitani, Kosuke Takeda, Hideto Kawaratani, Yasushi Okura, Kosuke Kaji, Akitoshi Douhara, Yasuhiko Sawada, Norihisa Nishimura, Kenichiro Seki, Akira Mitoro, Junichi Yamao, Hitoshi Yoshiji

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript No: 27269

We thank the reviewers for the insightful comments on our manuscript. We have carefully studied the items they have raised. Please find enclosed our point-by-point answers to their questions and items. The text in the manuscript has been revised in line with the reviewers' suggestions essentially improving the manuscript. All changes in the revised manuscript are highlighted in red.

Comments from reviewer 1:

My only concern is how do the authors compare the therapuetic effects of demethylating for the treatment of liver fibrosis to that of Green Tea Extract (GTE) and the oxymatrine on preventing hepatic fibrosis and ant-fibrotic effects of Gantai capsules and several other agents? I therefore, have no further comments on this paper except to approve it for its publication.

We appreciate the reviewer's positive assessment of our manuscript. In the future, using hepatic stellate cell *in vitro*, we would like to compare the therapeutic effect of demethylating agents on liver fibrosis development to that of other agents.

Comments from reviewer 2:

The study is original and well designed. My recommendation is accepted.

We thank the reviewer for his positive assessment of our manuscript.

Comments from reviewer 3:

1. *Can the authors comment on the use of the CDAA diet model – while this model replicates the fat accumulation and fibrosis seen in human NASH, the metabolic profile is different to that seen in humans. Would use of a different NASH model influence Agtr1a methylation?*

As the reviewer points out, the CDAA model replicates histological changes similar to those in human NASH but different from human NASH, obesity, glucose intolerance, and insulin resistance, which are not observed in this model. Unfortunately, we did not analyze *Agtr1a* methylation in a different NASH model. We newly described the advantages and disadvantages of our study using the CDAA model in the revised manuscript (page 12, lines 19–25).

2. *The main finding of the study is that methylation increases in CDAA-fed animals but this finding is not statistically significant. Can the authors comment on the high variability seen in their results – is the animal model variable or the methodology? Was the study powered sufficiently to detect changes? Why are different group sizes used for each group?*

We thank the reviewer for pointing out this important issue. We consider that the high variability observed in the results depends on individual differences in rats and tissue heterogeneity in each sample, both of which are hardly avoided. We have added this comment in the discussion section of the revised manuscript (page 13, lines 1–8). As for methodology, methylation-specific PCR is a quantitative and highly reliable method. Regarding the different sample sizes in each animal group, sample sizes for group i) -

iv) were 5, 12, 10, and 12, respectively, at the beginning, but two animals (one for CSAA-diet for 8 weeks and the other for CSAA-diet for 12 weeks) were dropped out because of entry in another experiment. We have added this comment in the revised manuscript (page 8, lines 7–9).

3. *Why are there no error bars or statistical analysis done for Figures 3 and 4? This implies the experiment was only performed once which is inappropriate for experiments using primary cell isolates.*

We thank the reviewer for raising this issue. We performed methylation and expression analysis in triplicate and presented data as the mean ± standard error in Figures 3 and 4 of the revised manuscript.

4. *No information is given on fibrosis in the model. Following 8 and 12 weeks of CDAA diet what fibrosis stage is reached?*

We thank the reviewer for this suggestion. We have added information about liver fibrosis in the CDAA model from our previous studies [Douhara A *et al*, Mol Med Rep, 11(3):1693, 2015 and Aihara Y *et al*, Hepatol Res, 43(11):1241, 2013] (page 12 lines 19–21). Correspondingly, reference #23 was added.

We once again thank all the reviewers for their helpful comments and suggestions. We hope that the revised manuscript will now be suitable for publication in the *World Journal of Hepatology*.

Yours sincerely,
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