March 24, 2013

Dear Editor,

Thank you very much for the comments from the reviewers. Please find enclosed our manuscript entitled, **"**Low endogenous IGF-1 levels contribute to diabetic colonic dysmotility mediated by decreased SCF". We have revised the manuscript based on the reviewers' suggestions, and tracked changes in the text in Word format (file name: 2135-review.doc) for the reviewers' convenience. The comments are addressed below in a point-by-point manner.

We hope the changes are satisfactory, and that you will now find the manuscript acceptable for publication. This manuscript has been edited and proofread by *Medjaden* Bioscience, Limited, to ensure the use of grammatically correct and idiomatic English.

(1) **This study is very similar to ref.-No.9. The authors should clearly describe the significance of this study and the difference compared with that of ref.-No.9.**

Answer: We appreciate the comments. The study cited as Ref. No.9 showed that: 1) there was a synchronous reduction in the number of ICCs, SCF mRNA levels and the smooth muscle marker, myh11, and its mRNA levels in gastric tissue from diabetic mice. In contrast, neuron-specific gene expression remained unchanged. 2) In organotypic cultures, compensation of IGF-1/insulin could have resulted in a synchronous decrease in myh11 mRNA levels, and an increase in SCF mRNA levels. 3) The IGF-1 receptor was studied in smooth muscle and neuronal cells, but not ICCs. Therefore, the authors could only conclude that reduced IGF-1 signaling in diabetes may lead to ICC depletion and its consequences by causing smooth-muscle atrophy and reduced SCF production.

The significant differences between that study and the current one are: 1) We showed evidence supporting the presence of dysfunction of smooth muscle contractility

in animals, which is more compelling evidence of myopathy than just a reduction of myh11 mRNA levels. 2) In cell culture studies, we demonstrated that IGF-1 has a direct effect on increasing SCF mRNA and protein levels, and this effect was mediated by ERK1/2 signaling. 3）Additionally, we found that endogenous IGF-1 levels in diabetic rat colonic tissues were decreased, and hyperglycemia may have been involved in initiating this change.

Therefore, the current study is not a repetition, but an extension of those described in ref. No. 9. This is now stated in the discussion on P12

(2) **In figure 4 C the authors used the PD98059 as an inhibitor of ERK1/2 pathway, and showed only the inhibition of the SCF induction by addition of IGF-1 in western blotting. So, the authors should also show the inhibition of p-ERK1/2 and these signaling by addition the band of western blot analysis in this panel.**

Answer： These comments are well taken. As recommended, we have now added the band from the Western Blot analysis of the effects of IGF-1 treatment on p-ERK1/2 and p-Akt proteins levels in SMCs. This now appears in Fig. 4.

(3) **In page 9, line 22, the authors should check and rewrite the number of "60%" and "44.19±25.73%".**

Answer： We appreciate the suggestion. We have now rewritten the results section as recommended.

(4) **In page 10, line 11, the authors should rewrite "AKt" to "Akt"**.

Answer： We have now corrected case of the abbreviation wherever it appears in the text.

(5) **In figure 5 B these bands is not clear and it is hard to see the difference expression levels of SCF protein, so the authors should modify this figure**

Answer： We appreciate the comment. As suggested, we have now modified the SCF bands in Figure 5B to make them clearer.

(6) **Specific comments Methods: specify number of experiments performed in all cases. Show them in the text and legends. Blots in figures are representative blots? How many were run in each case? Please specify in the legends of the figures**.

Answer: We appreciate the comments. All the experiments in this study were performed in triplicate or quadruplicate. Western Blot images shown in the figures are representative blots. Six to eight samples were run in each case. The numbers of runs are now stated in the figure legends as suggested.

(7) **Discussion: Homozygous IGF-I receptor KO mice do not survive after birth. No studies on intestine have been performed on these animals. Mice with low circulating IGF-I due to liver IGF-I deletion have normal levels of IGF-I in the rest of tissues. Thus, whether there is redundancy or not in the GI tract to compensate IGF-I loss cannot be based on these findings.**

Answer: We appreciate and agree with the comments. Accordingly, we have now deleted that text from discussion. Instead, we have now cited references to support the view that endogenous IGF-1 in the GI tract plays a central role in the growth and development of visceral and vascular smooth muscle.

(8) **Methods, Western blots: Ready Gel in 12% Tris-HCl? You mean 12% gel?**

Answer: We appreciate the correction. We meant a 12%polyacrylamide gel. This has now been clarified by revising the sentence in the methods section on P. 7.

(9) **Results: values shown in figures may be omitted in the text; i.e.: glucose levels, body weights….etc**

Answer: As recommended, the values shown in the figures have now been deleted from the results.

(10) **Instead of referring that "IGF-I induces expression of SCF" when measuring protein levels by WB rather refer to "IGF-I increases levels of SCF" as no direct measurement of expression of SCF were done**

Answer: As recommended, we have now corrected this in the text.

(11) **Legends Figure 3: include time at which SCF and IGF-I levels were measured after IGF-I siRNA . Figure 4A: include time after IGF-I addition**

Answer: We appreciate the comments. The legend for Figure 3 has now been modified as follows: A: SCF mRNA levels in colonic SMCs seventy-two h after transfection with IGF-1 siRNA, (each bar represents the mean ± standard error of 3 independent experiments). a. $P<0.05$, and b, $P<0.01$ compared with controls; and c, $P<0.05$ and d, $P<0.01$ compared with negative controls. B: SCF protein levels in SMCs after IGF-1 knockdown for 72 h (the figure is representative of 3 experiments).

The legend for Figure 4A was modified as follows: IGF-1 effects on the expression of SCF as a function of concentration after treatment with IGF-1 for 24 h. b, $P<0.01$ compared with 0 ng/ml IGF-1; c, $P<0.01$ compared with 50 ng/ml IGF-1.

(12) **some grammatical errors still existed. For example, the sentence "It is well established that ICCs survival and function depend on the activation of c-kit, a receptor tyrosine kinase integral to ICCs, by SCF (kit ligand) produced locally within the tunica muscularis" is not smooth. Hence, the language should be revised by the native speaker.**

Answer: We appreciate the comments. This sentence has been rewritten to read, "It is well established that ICC survival and function depend on the activation of c-kit, a receptor tyrosine kinase integral to ICCs. SCF, as kit ligand, is produced locally within the tunica muscularis".

As mentioned above, the manuscript has been reviewed and modified by *Medjaden* Bioscience, Limited, to ensure the use of grammatically correct and idiomatic English.

References and typesetting have now been corrected

Thank you again for considering our manuscript. We hope that it is now suitable for publication in

the *World Journal of Gastroenterology*.

Sincerely yours,

Wang Yun, PhD
Department of Gastroenterology
First Affiliated Hospital of Nanjing Medical University
300 Guangzhou Rd
Nanjing
PR China
Fax: +86-25-83674636
E-mail:nj_wendy@163.com