# Format for ANSWERING REVIEWERS

February 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7874-review.doc).

Title: Mesothelin promotes cell proliferation in the remodeling of neonatal rat pancreas

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**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 7874

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been update

2 Revision has been made according to the suggestions of the reviewer
(1)Reviewer 1:

"I believe that the work I have reviewed has important implications in the future as it shows the ability of mesothelin as mediator stimulating the proliferation of pancreatic cells and their possible use in the future. While the exact mechanism of action is not described, no contradictions are observed in utilization and effect relationship. We also observe the presence and action in normal, non-cancerous cells, which also has a high value because it raises the value of the protein but not as oncologic marker, but as a marker of damage and possibly help signal. It would be interesting to establish the relationship between mesothelin, damaged normal cells, and local proliferation of cells but also migration of progenitor cells from bone marrow. This mechanism would put the stamp seal help. The work not only stands out for its findings but also by the detailed methodology. Congratulations !"

**Replies to reviewer1:**

Thank for the comments of reviewer 1 on our study. Though the exact mechanism of action is not described in present study, we will continue to explore the exact mechanism in the next plan.

(2)Reviewer 2:

"In this study the authors investigated the role of mesothelin in development of endocrine pancreas in neonatal rats. One major issue is that the cell line INS-1 is a tumor cell line (insulinoma cell line), not a "normal cell" cell line. The signaling pathways in tumor cells and in normal islets are different, and therefore the results from tumor cell lines can not be used to "explain" observations in normal islets. There are also multiple grammatical errors."

**Replies to reviewer2:**

Thank you for the advice of reviewer 2 on our study.

① The main comments of reviewer 2 on our study is the cell line INS-1 is a tumor cell line (insulinoma cell line), not a "normal cell" cell line. And the cells can not be used to explain the observation in normal islets.

Till now, a lot of studies have been carried out on INS-1 cells for exploring the basic function of beta cells. Terri N. Iwata *et al.* have demonstrated that the transcriptional co-regulator host cell factor-1

(HCF-1) plays critical roles in pancreatic beta cell function[1]. After downregulation of HCF-1 expression in the INS-1 pancreatic β-cell line, they found decreased cell proliferation, decreased glucose-stimulated insulin secretion and decreased expression of the critical beta cell transcription factior Pdx1. otherwise, they also demonstrated that HCF-1 and E2F1 co-localize to the Pdx1promoter in INS-1 cells. *Li Chen. et al* have demonstrated that orexin A affects INS-1 rat insulinoma cell proliferation via orexin receptor 1 and the AKT signaling pathway related with orexin A [2]. Auffret J. *et al* have demonstrated that defective prolactin signaling impairs pancreatic β-cell development during the perinatal period. In INS-1 cells, they found that PRL stimulated leucine incorporation and S6 kinase phosphorylation, supporting a role for β-cell mTOR signaling in PRL action. Then, in the Goto-Kakizaki (GK) rat, they found IGF-II, a PRL target, was decreased [3]. In conclusion, the INS-1 cells can be adopted for exploring the basic function of beta cell and investigating the signaling pathway.

In our present study, we explored the effect of mesothelin on beta cell proliferation and cell apoptosis after upregulation and downregualtion of mesothelin in INS-1 cells. Exogenous over-expression of mesothelin could promote cell proliferation, cell colony formation and enhanced cell resistance to apoptosis of INS-1 cells. Down-regulation of mesothelin made no difference in cell proliferation and apoptosis compared with that in the control group. After an injection of Adenvirus-mesothelin in vivo, the number of small islets and the total number of islets are both increased and the expression of PCNA was decreased in day 7 and day 14 compared with Ad-EGFP group. By the studies in vivo and in vitro, we demonstrated that mesothelin could promote β cell proliferation in the remodeling stage of the neonatal rats. Mesothelin may have an important role in the remodeling of endocrine pancreas in neonatal rats.

② Multiple grammatical errors have been corrected.

(3)Reviewer 3:

"Authors demonstrated that overexpression of mesothelin could promotes beta cell proliferation in vitro while its down-regulation increased the islet number in neonatal rat pancreas. 1. Line 304-306 (p11) are confusing. Why did authors suspect the increased number of small islets in spite of down-regulating mesothelin? Because mesothelin could promote beta cell proliferation, it was more reasonable to expect the decreased number of islets. 2. There seems to be a typo (line 93, p4)."

**Replies to reviewer3:**

Thank you for the advice of reviewer 3 on our study.

① The remodeling period of pancreas is the 2–3 weeks after birth for the islet remodeling and maturation.[4]. Many major developmental changes occur during pancreatic remodeling stage, including β-cell apoptosis, replication and neogenesis.

L. Scaglia. *et al.* has reported that in the remodeling of endocrine pancreas, the β cell proliferation ratio would be decreased [4]. Islet formation in the neonatal pancreas may occur by fission of elongated structures composed of β cells and surrounding α cells, following the contiguous proliferation and branching of endocrine cells into cord-like structures in the newborn mouse pancreas. A large islet would be cut into several small islets in the neonatal rats [5]. Then we hypotheses that due to the decreased β cell proliferation ratio, the small islet number would be increased.

In fig4, we adopted Image J to measure the number and size distribution of islets according to the fluorescent images of insulin and glucagons. We showed the overall distribution of islets (including small clusters of β cells) in day 7, day 14, 1 Mo pancreas after downregulation of mesothelin and we found that the number of small islets in Ad-mesothelin group is more than that in Ad-EGFP group in day 7 and day 14. Otherwise, the total numbers of islet are also increased in Ad-mesothelin group than Ad-EGFP group in day 7 and in day 14 (after downregulation of mesothelin in the remodeling period of neonatal rat pancreas). This part has been inserted into the results of fig4.

The reviewer had a misunderstanding of the Line 304-306 (p11) in the previous manuscript. We are sorry for the misunderstanding due to unclear descriptions for explaining why we suspect the PCNA protein. The immunohistochemisty staining of PCNA protein is used for detecting the decreased cell proliferation after downregulation of mesothelin in pancreas. PCNA expression was

decreased in 7 days and 14 days of Ad-mesothelin group when compared with Ad-EGFP group respectively. We apologized that we did not express our purpose completely.

In conclusion, the beta cell proliferation ratio would be decreased after downregulation of mesothelin. Due to the decreased cell proliferation, the small islet number and the total islet number are both increased in day 7 and day 14 pancreas after downregulation of mesothelin. And the decreased cell proliferation has also been demonstrated by immunohistochemisty staining of PCNA. PCNA expression was decreased in 7 days and 14 days of Ad-mesothelin group when compared with Ad-EGFP group respectively.

② A typo (line 93, p4) has been corrected.

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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**Reference**

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2 Chen L, Zhao Y, Zheng D, Ju S, Shen Y, Guo L. Orexin A Affects INS-1 Rat Insulinoma Cell Proliferation via Orexin Receptor 1 and the AKT Signaling Pathway. *Int J Endocrinol* 2013; **2013**: 854623 [PMID: 24382962 PMCID: 3871501 DOI: 10.1155/2013/854623]

3 Auffret J, Freemark M, Carre N, Mathieu Y, Tourrel-Cuzin C, Lombes M, Movassat J, Binart N. Defective prolactin signaling impairs pancreatic beta-cell development during the perinatal period. *Am J Physiol Endocrinol Metab* 2013; **305**(10): E1309-1318 [PMID: 24064341 PMCID: 3840213 DOI: 10.1152/ajpendo.00636.2012
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4 Scaglia L, Cahill CJ, Finegood DT, Bonner-Weir S. Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. *Endocrinology* 1997; **138**(4): 1736-1741 [PMID: 9075738]

5 Miller K, Kim A, Kilimnik G, Jo J, Moka U, Periwal V, Hara M. Islet formation during the neonatal development in mice. *PLoS One* 2009; **4**(11): e7739 [PMID: 19893748 PMCID: 2770846 DOI: 10.1371/journal.pone.0007739]