Format for ANSWERING REVIEWERS

January 22, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6645-review.doc).

Title: The pathophysiological roles of Pim-3 kinase in pancreatic cancer development and progression

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6645

The manuscript has been improved according to the suggestions of reviewers and major revisions are underlined in the revision.

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) **Reviewer 1**

 # 1. I would suggest that a native English speaker review and edit the manuscript. While it is generally readable, there are some syntax errors that detract from the readers' experience.

Response - The manuscript has been extensively edited by an English editing company and we attach the certificate from the company.

#2. To help the reader, consider re-organizing section 5 such that there are 4 subheadings, as you have indicated in the introduction of section 5: survival signaling, regulation of cell cycle progression, regulation of protein synthesis. That paragraph on page 11 is confusing, as you outline four activities involved in carcinogenesis, but the last sentence then says 'two aspects' of Pim-3 effects on carcinogenesis.

Response – In response to the recommendation, we modified the paragraph of page 11 as follows: "Pim-3 can contribute to cancer development and progression by acting on tumor cells and tumor microenvironments. The primary activities of Pim-3 on tumor cells include the delivery of survival signaling, the regulation of cell cycle progression, protein synthesis, and Myc transcriptional activity (Figure 3). In addition to its effects on tumor cells, Pim-3 can have profound impacts on tumor microenvironments, especially neovascularization process (Figure 3). In the following sections, we will discuss the roles of Pim-3 in carcinogenesis, with a focus on these two aspects."

#3. The paragraph on K-ras mutation and activation is under the 'tumor microenvironment' subheading, but also contains information about Pim-1 expression and prostate cancer. This should either be clarified or eliminated, so as not to focus on prostate cancer.

Response – We deleted the paragraph on K-ras mutation and activation in response to the

recommendation.

#4. Some of the material does not directly pertain to pancreatic cancer, and could potentially be eliminated. An example is the first paragraph of page 11, with the discussion of hippocampul induction. Another paragraph that could be cut is the first one on page 14, discussing hypoxic microenvironments and HIF1a. Only the last sentence mentions Pim-3, which has nothing to do with the paragraph on hypoxia.

Response – We deleted the first paragraph of page 11 and the first paragraph of page 14 in response to the recommendation.

(2) Reviewer 2

#1. Provide an abbreviation list to explain the detail meanings of most abbreviations appeared in the review.

Response – We indicated the abbreviation at their first appearance in both the abstract and the text, except standard abbreviations.

#2. Are there experimental evidences to support the argument in page 10, lines 1 and 2?

Response – We added the following paragraph in page 10. "Similarly, the transfection with Pim-3 shRNA reduced G1 population of human pancreatic cancer cells compared with the cells transfected with scramble shRNA. Moreover, a small-molecule Pim-3 kinase inhibitor markedly retarded in vitro growth of human pancreatic cancer cell lines by inducing G2/M arrest, suggesting a potential role for Pim-3 in cell cycle progression. Consistently, cell cycle progression is accelerated in hepatocytes of transgenic mice, which express human Pim-3 cDNA selectively in hepatocytes and downregulation of Pim-3 decreased the amounts of Cdc25C, cyclin B1, and phospho-p21 (Our unpublished data). Thus, Pim-3 can promote cell cycle progression and eventually contribute to carcinogenesis by modulating the functions of these regulatory molecules involved in cell cycle progression."

#3. What was stated in page 11, paragraph 4, second sentence, was not shown in figure 3.

Response – We modified the second sentence in page 11, paragraph 4 as follows: "In addition to its effects on tumor cells, Pim-3 can have profound impacts on tumor microenvironments, especially neovascularization process (Figure 3)"

#4. How did Pim-3 affect Wnt/β-catenin pathway is not clearly stated in the 3rd paragraph of page 12, there the conclusion that "Pim kinases including Pim-3 may sustain cell survival by modulating Wnt/β-catenin …" is lack of support.

Response – We modified the 3rd paragraph of page 12 as follows: "Cell survival can be regulated by Wnt/β-catenin and Stat3 signaling pathways. An integrative molecular screening by using siRNA identified Pim-3 as a new regulator of Wnt/β-catenin signaling. Thus, Pim-3 can positively regulate the Wnt/β-catenin signaling pathway in the colorectal cancer cell lines (DLD-1 and SW480). Moreover, Pim-3 is a positive regulator of Stat3 signaling in the prostate cancer cell line (DU-145) and in the pancreatic cancer derived cell line (MiaPaCa2). Thus, Pim-3 canpromote cancer cell survival by modulating Wnt/β-catenin and/or Stat3 signaling pathways."

#5. There is not enough evidence to make the claim in the last sentence of page 15, 2nd paragraph

Response – We deleted the second paragraph of page 15

(3) Reviewer 3

#1. The paper is a bit long. It could be more easily readable if shortened.

Response – We deleted the part of the second paragraph of page 7, the first paragraph of page 11, the fourth paragraph of page 12, the second paragraph of page 14, and the second paragraph of page 15.

#2. The paper seems more a general and descriptive review about Pim family kinase rather than a targeted review on the roles of Pim-3 in pancreatic cancer development.

Response – We generally modified the manuscript to focus on the roles of Pim-3 in pancreatic cancer development.

#3. Moreover the Authors often extrapolate the mechanism of action of Pim -3 kinase from the one of Pim -1 and -2, without a direct evidence (in the introduction actually justifying it with their similar - but not identical - gene sequence). In detail, on page 7, second and third paragraphs, they refer only to the Pim -1 effects, concluding that Pim -3 affects cell cycle and carcinogenesis only on the basis of sequence similarity with Pim -1. Only as an example, they infer the role of Pim -3 only on the basis of similarity to Pim -1, also on page 12, third paragraph and on page 15 (last line).

Response – We deleted the part of the second paragraph and modified the second paragraph of page 7 as follows: "Similar to Pim-1, Pim-3can autophosphorylate some of their serine residues but whether this has any functional significance is yet to be elusive. Moreover, Pim-1 and Pim-3 have been shown to bind to the serine/threonine protein phosphatase 2A (PP2A), resulting in their dephosphorylation, ubiquitination, and proteasomal degradation.

Response – We modified the third paragraphs of page 7 as follows: The relevant analysis for the structure of human Pim-3 mRNA indicates that 3′ UTR of the Pim-3 gene harbors multiple binding sites for miRNAs (www.ebi.ac.uk; www.microrna.org). It will be interesting to know whether Pim-3 translation can be regulated in a similar manner."

Response – We deleted the third paragraph of page 12.

#4. The Authors often discuss the different roles of Pim-3, but not always specifically focusing on the pancreatic carcinogenesis. For example, in the abstract, lines 10-12, they expound the effects of Pim-3 on the liver. The same on page 5 and, again, in the chapter 4 (Biological functions) where the role of Pim-3 on pancreatic cancer development comes only after 3 pages and it is explained only in a single paragraph (page 11 second paragraph).

Response – We deleted the sentences in lines 10-12 of the abstract. We described the general biological functions of Pim-3 followed by the roles of Pim-3 in pancreatic cancer.

#5. The purpose of the manuscript was not clearly stated because the Authors never explained why they want to focus on Pim-3 protein and in particular on its functions on pancreas carcinoigenesis. A sentence should be added to clarify the aim.

Response – We modified the section of introduction, the first and the second paragraph of page 3 as follows: "Thus, a novel molecular targeted therapy will be a required therapeutic option for human pancreatic cancer treatment." And "Pim-3 kinase has essential roles in the regulation of signal transduction cascades. Moreover, its expression is enhanced in human pancreatic cancer cell lines and blocking of its expression induced apoptosis and drug resistance in human

pancreatic cancer."

#6. In conclusion, I think the manuscript should focus more closely on the topic mentioned in the title.

Response – We generally modified the manuscript to focus on the roles of Pim-3 in pancreatic cancer development.

Specific comments:

#1. Introduction, second paragraph: the Authors wrote "Provirus integrating site Moloney". Consider writing ": Provirus integration site for Moloney…"

Response – We replaced the "Provirus integrating site Moloney" with "Provirus integration site for Moloney".

#2. Page 4, line 4 The Authors wrote "In this Reviwe, we aim to highlight the pathophysiological roles of Pim-3 in development and progression of caner, particularly pancreatic cancer. Please correct Reviwe with review and caner with cancer.

Response – We replaced the Reviwe with review and caner with cancer.

#3. Page 4, line 8 please substitute "cancer therapy" with "antineoplastic therapy".

Response –We replaced the "cancer therapy" with "antineoplastic therapy"

#4 . Page 10 lines 18-22 : missing reference (probably # 33).

Response – We cited the reference # 33 in Page 10 lines 18-22

#5 . Page 12 , last paragraph : the sentence refers to a work already published by themselves (ref# 76) but the results of that work concerned the effects on the liver and not on the pancreatic effects.

Response – We deleted the last paragraph of page 12.

#6. Page 17 , Chapter 7 , last sentence: It should be better if the Authors write " Studies focusing ON THESE aspects" rather than "the study ... " .

Response – We replaced the "the study" with "further studies on these aspects".

(4) Reviewer 4

Major comments:

#1. Some parts of the paper are citated insufficiently, e.g. page 4/5 concerning the sequence similarity. Also on page 14 there is no citation for the statement ? Pim-1 expression is increased under hypoxia in pancreatic cancer cells, independently of HIF-1 a". Here, some of the statements are quite speculative.

Response – We modified the "sequence similarity" as "sequence similarity (Figure 1 and NCBI Reference Sequence: NP_001001852.2)"

Minor comments:

#1. HIF-1a is termed hypoxia ?inhibitor" factor, which is uncorrect, it should correctly be hypoxia inducible factor 1a.

Response –We deleted the second paragraph about under hypoxia condition.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Yours sincerely,

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