ANSWERING REVIEWERS

April 30, 2014

Dear Prof. Lian-Sheng Ma,
President and Editor-in-Chief *"World Journal of Gastroenterology"*

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript No. 9047 WJG.doc).

Title: Claudin 1 mediates TNFα-induced cell migration in human gastric cancer cells

Author: Atsushi Shiozaki, Hiroki Shimizu, Daisuke Ichikawa, Hirotaka Konishi, Shuhei Komatsu, Takeshi Kubota, Hitoshi Fujiwara, Kazuma Okamoto, Daisuke Iitaka, Shingo Nakashima, Yoshito Nako, Mingyao Liu, Eigo Otsuji

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9047

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

2 Revision has been made according to the suggestions of the reviewer
Comments from Reviewer *00503458*:
In this paper the authors investigated the role of Claudin 1 in the regulation of genes involved cell migration and TNF-α-induced gene expression in human gastric adenocarcinoma MKN28 cell line. The knockdown of Claudin 1 significantly inhibited cell proliferation, migration infiltration and increased apoptosis. Microarray analysis reveled that Claudin-1 knockdown modulate the expression of 245 genes. Pathway analysis showed that the top-ranked molecular and cellular function was the cell migration related pathway and that TNF-α and NFkB were the top-ranked upstream regulators related to Claudin-1. The involvement of Claudin 1 in tumorigenesis and in tumor progression has been widely documented particularly in gastric cancer where it has also been reported that up-regulation of Claudin 1 is related to transformation in invasive and metastatic cancer. The same authors have recently showed that the expression of Claudin 1 was increased in upon TNF-α treatment of A549 lung carcinoma cell line. Due to the previous studies already published this manuscript lacks of originality however, the study is well conceived and the experimental plan has been rigorously conducted. The only concern raised by this reviewer is the use of only one cell lines throughout the study. The validation of the results in at least another gastric adenocarcinoma cell lines could reinforce the conclusions.
Response:
Thank you for the precious comments and suggestions. According to the reviewer's comment, we examined several gastric cancer cell lines, NUGC4, MKN45 and Kato-III to determine changes of claudin 1 expression and migration induced by TNFα stimulation (Fig. 5B, 6B). We revised the Result part (page 12), as follows. Further, we added new Fig. 5B, 6B, and Figure legends.
"The basal expression level of claudin 1 was increased in a time-dependent manner at 24 and 48 h, which was further increased by TNFα stimulation in MKN28 cells (Fig. 5A). Similar trends that the expression level of claudin 1 was increased by TNFα stimulation were found in several gastric cancer cell lines,

including NUGC4, MKN45 and Kato-III (Fig. 5B).” the Result part (page 12)
“TNFα treatment increased cell migration in MKN28 cells (Fig. 6A). Similar trends were found in several gastric cancer cell lines, including NUGC4, MKN45 and Kato-III (Fig. 6B).” the Result part (page 12)

Comments from Reviewer *01197938*:
Authors described the role of claudin1, one of the gap junction components, in gene regulation and cellular kinetics in gastric carcinomas. They identified a tumor progression role of claudin 1 in MKN28 cell line, while similar phenomena have been reported in other malignant cell lines. This reviewer suggests the number of issues, as noted bellow; TNF-a signals are reportedly dominated by NF-kB transcriptional systems. Does silencing of claudin1 alter NF-kB signals? Subcellular distribution of claudin1 during expression changes is needed to be investigated. How claudin1 modulate gene expression? This should be mentioned. Claudin1 forms gap junctions in normal epithelium and loss of claudin1 facilitates epitherial transformation. Conversely, over-expression of claudin1 in cancer cell lines augments their malignancy in some instances. It seems contradictory. Why such difference occurs?
Response:
Thank you for the precious comments and suggestions. We have performed microarray and Ingenuity Pathway Analysis, and showed that NFκB was the top-ranked regulator related to claudin 1 (Table 2), suggesting that silencing of claudin1 alters NF-kB signals.
Further, According to the reviewer’s comment, we examined the change of subcellular distribution of claudin1 induced by TNFα stimulation using immunofluorescent staining and confocal microscopy. We revised the Result part (page 12), as follows. Further, we revised the Materials and methods part (page 9-10), and added new Fig. 5C, and Figure legend.
“Furthermore, immunofluorescent staining demonstrated that TNFα-induced claudin 1 protein expression was mainly found in the cytoplasm of MKN28 cells (Fig. 5C).” the Result part (page 12)
In addition, we discussed about the different functions of claudin1 in the Discussion part (page 14), as follows.
“Our recent report showed that TNFα strongly increased claudin 1 expression in the cytoplasm in human lung cancer A549 cells, and that knocking down of claudin 1 blocked the TNFα-induced gene expression and cell migration [20]. Similarly, in the present study, TNFα increased claudin 1 expression in the cytoplasm in human gastric cancer MKN28 cells, and knocking down of claudin 1 blocked the TNFα-induced gene expression and cell migration in human gastric cancer cells. Generally, claudin 1 participates in cell-to-cell adhesion as TJ proteins, and its down-regulation may promote cell migration. However, our findings showed TNFα-induced claudin 1 is mainly in cytoplasm, and is involved in TNFα-induced gene expression [20]. In addition, considering that many of these claudin 1 dependent genes are related to cell movement and morphology, claudin 1 may mediate TNFα-initiated cell migration with multiple mechanisms.” the Discussion part (page 14)

Comments from Reviewer *00077679*:
This article by Shiozaki A et al. reported “Claudin 1 plays important roles in cell migration and TNF signaling in human gastric cancer cells”. They reported that claudin 1 knock down significantly inhibited cell migration and invasion in gastric cancer cells. And the down-regulation of claudin 1 changed the expression level of TNF-alpha signal. This article shows sufficient quality to publish in WJG, but before publishing the authors need to add some data. General comments: The biggest issue is that the author used only one cell line, MKN28, and reported. If we report the results of experiment with a type of cancer, at least 5 cell lines are necessary. Please add 4 more gastric cell line for this experiment. Minor point: 1) Why the author used Claudin 1? claudin 1 is better.

Response:

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"TNFα treatment increased cell migration in MKN28 cells (Fig. 6A). Similar trends were found in several gastric cancer cell lines, including NUGC4, MKN45 and Kato-III (Fig. 6B)." the Result part (page 12)

Further, we change "Claudin 1" to "claudin 1", in the whole manuscript.

Comments from Reviewer *00004011*:

It is a well documented and very interesting manuscript.

Response:

Thank you for the precious comment.

Comments from Reviewer *01939901*:

I hope the paper will be published to guide more researchers.

Response:

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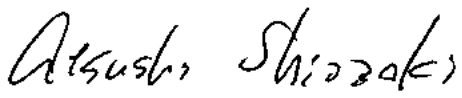
3 References and typesetting were corrected

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

Sincerely yours,



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