September 21, 2014

Dear Prof. Ma

Please find enclosed the edited manuscript in Word format (file name: Terao et al WJG Text revise).

Title: Fucosylation is common type of glycosylation in pancreatic cancer stem cell-like phenotypes

Author: Naoko Terao, Shinji Takamatsu, Tomomi Minehira, Tomoaki Sobajima, Kotarosumitomo Nakayama, Yoshihiro Kamada, Eiji Miyoshi

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 13060

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

Response to the comments of reviewers

We thank the reviewers for the positive assessment of our manuscript and for identifying areas that required corrections and/or modification. The red-colored text in the revised manuscript is the corrected/modified parts. All line numbers mentioned in each response to each comment refer to the small-size numbers that appear on the left margin of the text of the revised manuscript.

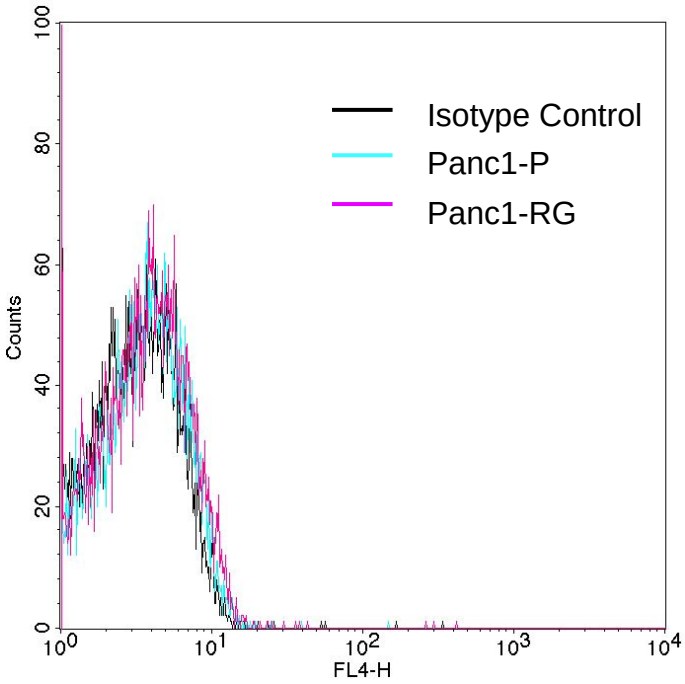
Response to the Reviewer 02544938

The manuscript of Terao et al "Fucosylation is a common type of…" is potential interesting report, but at this stage this is a very preliminary study. The main point of authors is that the fucosylation of PDAC cells can be a marker of CSC. From the present work this statement is not evident for me. The author used a very unspecific marker of CSC – CD24 and CD44. The authors must add min. CD133 expression and characterized the CSC as triple (CD24+CD44+CD133+) cells. Another important point is that the authors did not present the sphere-forming

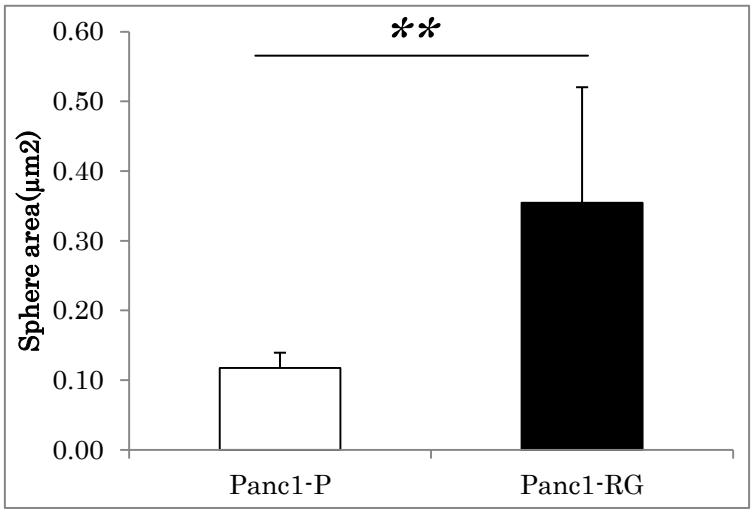
assay for the Panc1-RG. It must be done to compare the functionality of CSC. The connection between IL-6 production and CSC state is not evident for me. The authors must perform once more the sphere-forming assay and measure the IL-6 production both in monolayer and sphere cells.

Thank you for the reviewer's valuable comments. As a reviewer suggested, CD133 is a well-known CSC marker. According to the reviewer's comments, we performed some additional experiments.

Firstly, we investigated the expression of CD133 in Panc1 cells (Panc1-P and Panc1-RG). As following figure demonstrated, CD133 was scarcely expressed in each Panc1 cells. Therefore, we adopted CD24 and CD44 without CD133 as the CSC markers in the revised manuscript. Thank you for your good suggestion.



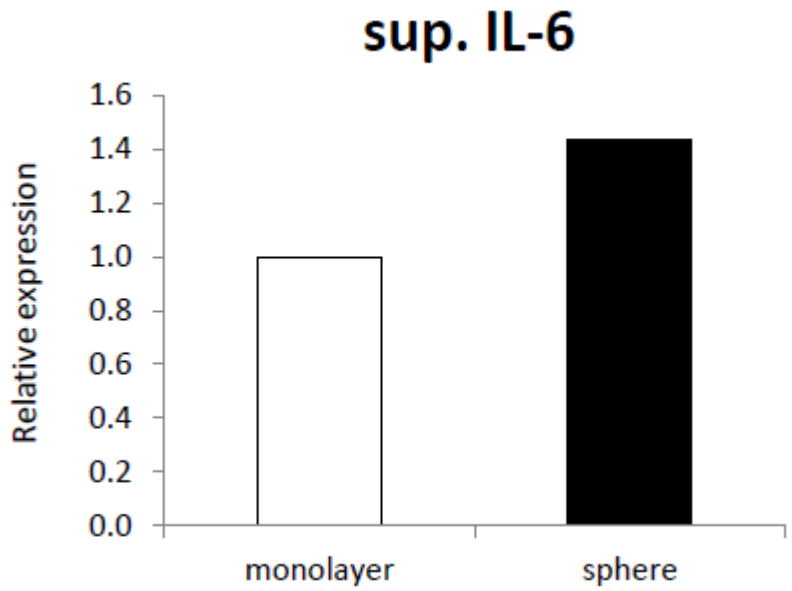
Secondary, we performed sphere-forming assay using Panc1-P and Panc1-RG. As following figure represented, sphere formation was significantly increased in Panc1-RG compared with in Panc1-P. We added some descriptions in our revised manuscript (page 13, line 301-5, and Figure 3E).



** $p < 0.01$

Thirdly, we investigated a relationship between IL-6 production and CSC status. As represented below, we found that sphere formation induced an increase in IL-6 production.

These results should emphasize the significance of our concept that IL6 is concerned with CSC phenotypes. We added some descriptions in our revised manuscript (page 14, line 340-3, and Figure 6C).



Response to the comments of reviewers

We thank the reviewers for the positive assessment of our manuscript and for

identifying areas that required corrections and/or modification. The red-colored text in the revised manuscript is the corrected/modified text. All line numbers mentioned in each response to each comment refer to the small-size numbers that appear on the left margin of the text of the revised manuscript.

Response to the Reviewer 02545023

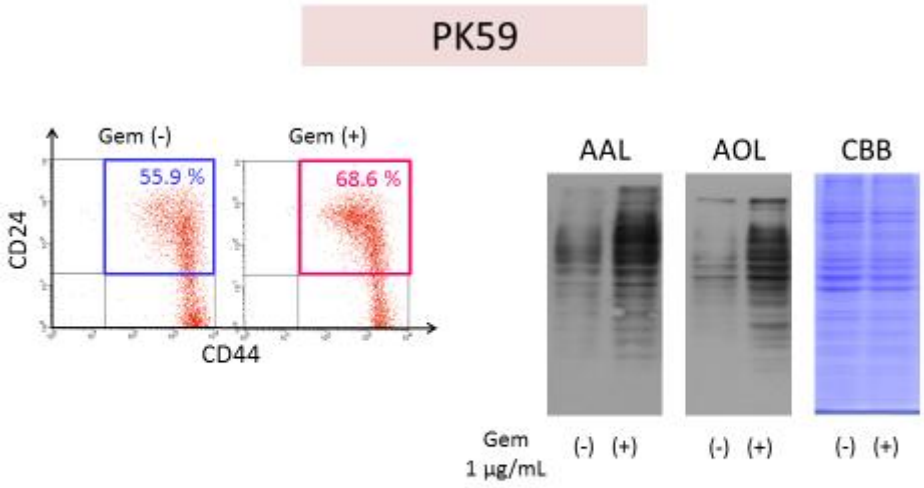
The manuscript by Terao et al reported an interesting finding that fucosylation is a common oligosaccharide modification in pancreatic cancer CSC-like cells, and increased cellular fucosylation is correlated with drug (such as gemcitabine) resistance. Therefore, the identification of fucosylated glycoproteins derived from pancreatic cancer cells could lead to novel biomarker development for anticancer drug resistance. Overall, the experiments were well done and properly interpreted. Comments/critics:

1). The patterns of lectin blotting in figure 2B, figure 3C and 3D are different which suggests that under different conditions Panc1 cells were able to develop different fucosylation. This phenomenon should be explained/discussed in the manuscript.

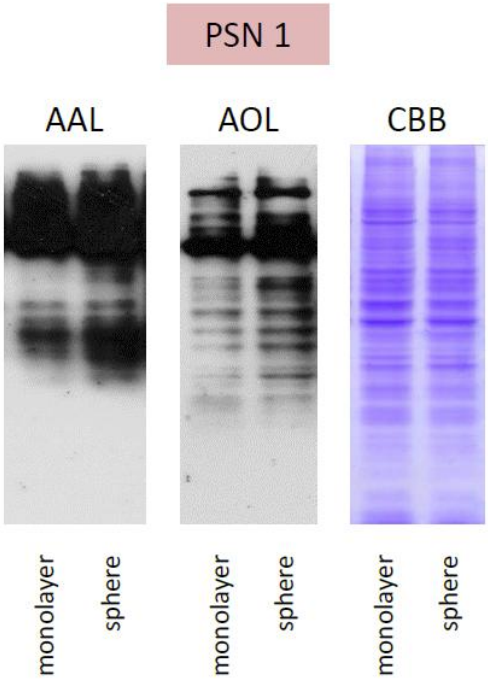
Thank you for reviewer's important suggestion. As a reviewer suggested, the fucosylation patterns in each figure are different. We think that these differences are dependent on the various conditions of cells in culture. It is a well-known fact that different conditions in culture should change the glycoprotein expression pattern. As CBB figures demonstrated the protein expression patterns were different in each condition. We added some descriptions in our revised manuscript (page 15, line 349-54).

2). The authors claimed that fucosylation is "a common type of glycosylation in the cancer stem cell-like phenotype of pancreatic cancer"; however, only ONE pancreatic cancer cell line, Panc1, was used in the whole study. The observations should be confirmed in multiple cell lines in order to draw such a conclusion. In addition, the findings/conclusion could be greatly strengthened, if possible, with using tumor tissues from pancreatic cancer patients or xenografted animal models.

Thank you for reviewer's valuable comments. According to the reviewer's comments, we investigated the fucosylation status in gemcitabine-treated pancreatic cancer cell lines (PK59, MIA PaCa-2, PSN-1, Capan-1, and BxPC-3) except Panc1. We found short-term gemcitabine treatment also caused concentration of CSC-like cells and enhanced fucosylation in several cell lines. We additionally showed the representative result in PK59 cells. We added some descriptions in our revised manuscript (page 12, line 271-8, and Figure 2E and 2F).



Next, we also investigated whether sphere formation enhance fucosylation in another pancreatic cancer cells. We investigated sphere-forming assay using five pancreatic cancer cell lines (PK59, MIA PaCa-2, PSN-1, Capan-1, and BxPC-3). Among these cell lines, sphere formation was succeeded in only PSN-1 cells. We further confirmed fucosylation was increased in sphere formation of PSN-1 cells. We added some descriptions in our revised manuscript (page 14, line 306-9, and Figure 3F).


PSN 1
AAL
AOL
CBB
monolayer
sphere
monolayer
sphere
monolayer
sphere