

August 25, 2012

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 12123-answering reviewers.doc).

Title: Impact of high glucose on the metastasis of colon cancer cells

Author: Cheng-Yao Lin, Chih-Hui Lee, Chien-Cheng Huang, Song-Tay Lee, How-Ran Guo, Shih-Bin Su

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 12123

I appreciate all the constructive comments of the reviewers and the opportunity to submit a revised version of my manuscript entitled, "**Impact of high glucose on the metastasis of colon cancer cells**".

I carefully revised my manuscript in accordance with all the comments raised. My point-by-point responses to the reviewers' comments are shown below. The corresponding changes in the revised paper are underlined.

The revised manuscript was also thoroughly checked and edited by a native English-speaking medical editor with specialist knowledge in this field to meet the language standards required by leading English language publications.

I hope that the revisions made have satisfactorily answered all the comments raised and that our paper is now suitable for publication in the *World Journal of Gastroenterology*.

Thank you in advance for considering our revised paper for publication. I look forward to hearing from you at your earliest convenience.

Sincerely yours

Cheng-Yao Lin, MD

Division of Hematology - Oncology, Department of Internal Medicine, Chi Mei Medical Center,
Liouying, Tainan, Taiwan

E-mail: d930827@mail.chimei.org.tw

The manuscript has been carefully revised in according to the suggestions of reviewers:

1. Format has been updated
 - The abstract had been revised to an informative, structured abstracts of no less than 246 words
 - A short summary was added as Core tip
 - A precise and simple "COMMENTS" section was added.
2. Revision has been made according to the suggestions of the reviewer

Reviewer 02551692

✧ ***In the third line of introduction it must be written "according to"***

Response

Thank you for helpful comment. We had change "according" as "according to" in our manuscript (page 5).

✧ ***In materials and methods: in the reagents section it is necessary to write what is "stattic and siRNA"; in Western blotting section the term PBS must be explained at the first appearance in the text***

Response

We had added full name of those abbreviations in the first appearance in the text as following: Stattic: STAT3 specific inhibitor; siRNA: Small Interfering RNA; PBS: phosphate buffer saline (page 7).

✧ ***The following sentences must be corrected to improve English language: In the section entitled "the effect of Stattic on the migration of CT-26 cells" of Results ,the sentence: "the higher the concentration of Stattic was, the fewer cells that migrated" In the second line of Discussion: "Adding STAT3 inhibitors, Stattic and siRNA, to a high-glucose environment, inhibited CT-26 cell migration and invasion. This indicated that the STAT3 signaling pathway is associated with regulating the effect of glucose on CT-26 cell migration and invasion".***

Response

We had revised the sentence as: "the number of the migrated cells reduced gradually with increasing concentrations of Stattic" in the result section (page 10) and "When STAT3 inhibitors, Stattic or siRNA, was added to a high-glucose environment, both glucose induced CT-26 cells migration and invasion were inhibited" in the discussion section (page 13).

✧ ***Figure Legends must be reviewed to use a correct and concise language.***

Response

As we are not native English speakers, we had gave our manuscript, include legends, to be revised by a native English-speaking medical editor.

✧ ***It is also important to report in the figure the technique used such as fluorescence or inverse microscope or western blotting.***

Response

Inverse microscope was used in this experiment and we had added it in the text (page 8).

✧ ***In the fig 9 it is not explained the effect of “stattic” on MMP9 and STAT3 expression.***

Response

In figure 9, we evaluated the expression of MMP-9 and STAT3 in high glucose environment (30mM glucose), both MMP-9 and STAT3 expressed higher than control (0mM glucose). When Stattic was added in the 30mM glucose environment, the higher expression of MMP-9 and STAT3 were diminished. The result explained that Stattic could inhibit high-glucose induced MMP-9 and STAT3 higher expression. Actin was a reference control.

We had revised the figure 9 legends to make more clear explanation (page 26).

- ✧ *Introduction: The introduction part is insufficiently focused, since too much is written about general problems of invasion and metastasis which is not primary focus of the here presented manuscript. The Introduction should be focused on the consequences of higher glucose level for invasion and metastasis. 26 literature citations are way too much for an introduction; not every argumentations requires 2-3 references support. Some citations are chosen not too well. For example: regarding the question of ECM degradation and angiogenesis especially in tumor patients are more targeted references available than ref. 7.*

Response

We had revised our introduction. We diminished some unnecessary sentences and put more focus on the glucose and cell invasion and metastasis. Meanwhile, the references were checked and total 18 literature citations are cited (page 5-6).

- ✧ *Materials and Methods: The methods part is comprehensible and clear. Here or in the discussion section the authors should provide a statement why they decided to apply a rat colon cancer cell line. There are multiple well examined human colon cancer cell lines available which would more suitable for this project.*

Response

Due to the available human colon cell lines in our laboratory are suspension-type cell lines that cannot evaluate migration function. So, we use rat-colon cell lines as our material. This limitation is important, so, we added this question in our discussion (page 15).

- ✧ *Results: Wound healing assay is a pretty rough assay for the evaluation of cell migration. Has been made sure that this phenomenon is not due to a different cell proliferation under the condition of a higher glucose concentration but a “true” migration? Was the cell count at the time of observation the same under every condition? Same question arises regarding the migration assay. It is well known from many cancer cell lines they show an increased proliferation under conditions of higher glucose levels (Beckner ME, J Natl Cancer Inst, 1990). So the results of the migration assay should correlate to the final cell count. This must be clarified to exclude the higher migration rate as a result of a higher proliferation rate.*

Response

This is also an important question; so, we calculated the cell count in glucose-free control group (0 mM) and different glucose-positive groups (10 mM, 20 mM, and 30 mM). The initial planted cell number is 5×10^4 . After 12-hours incubation, the mean cell counts in each group were 23×10^4 ; 25×10^4 ; 26×10^4 ; and 24×10^4 in 0/10/20/30 mM glucose respectively. The cell numbers indeed increased slightly in glucose-positive groups than glucose-free control group. But the difference was little. Besides, the cell numbers were not different in different glucose concentrations (10/20/30 mM). So, we thought the migration and invasion in glucose-positive

environments is contributed by increased cell activity rather than cell proliferation

- ✧ *In figure 1 the authors should explain why with 10mM glucose the wound strip became wider instead of smaller.*

Response

We revised our migration pictures and reconstructed in figure 1. The wound strip in 10 mM glucose was not wider (figure 1, page 27).

- ✧ *In general the data regarding cell staining (with what) and which magnification has been applied for the recordings (see figure 2).*

Response

The cell was stained by hematoxylin and it had been mentioned in the Material and Methods section (page 8). As the diverse magnification in every figures, the size of Scale Bar had added in figure 1~6 (page 27 ~ 32).

- ✧ *The Material and Methods part or the legends to the figures should mention the number of independent measurements from which the results originated.*

Response

The repeated measurement number of each test is 6 (N=6). We had mentioned it in figure 1~10 (page 27 ~ 36).

- ✧ *Discussion: Discussion should be more focused. The situation remains that regarding the questions of diabetes and cancer, especially colorectal cancer many open questions remain. As an example: is there a direct connection or a secondary connection caused by obesity and/or changed life style – see Giovannucci et al Diabetes Care, 2010. This unclear situation should be shown in the discussion.*

Response

About the issues, we added one paragraph in discussion to explain the unclear situation between DM and cancers risks (page 13).

- ✧ *The same applies for MMP-9 whose position should be evaluated in a more critical fashion. There are evaluations available (Koskensalo S BMC Clinical Pathology 2012) that not only the overexpress of MMP-9 may be a negative prognosis factor but the missing expression of MMP-9 may be a negative prognosis factor as well. Furthermore should it be pointed out that MMP-9 in colorectal cancer more often come from tumor surrounding connective tissue cells than from primary colorectal cancer cells and may have an inverse influence on the angiogenesis (see Taguchi et al PLOS one ,2014).*

Response

Thank you for kindly remind us those questions. In true, our results cannot answer those controversies; so, we mentioned it in our discussion to let readers understand those unresolved issues. We expect us and who are specialized in this field could keep study to answer those questions in the further (page 15-16).

Reviewer 02537353

✧ *It's very important to introduce in the several experiments another monosaccharide as control*

Response

Our study is focused in the relation between diabetes and colorectal cancer, and glucose is the major monosaccharide considered to be associated with diabetes. So, we did not introduce other monosaccharide in our manuscript. Even though, we used mannitol as control to evaluate its impact on the STAT3 and MMP-9 expression in our study. We also mentioned it at result “*The effect of glucose on STAT3 and MMP-9*” (page 11).

✧ *There are several English mistakes, I suggest the revision of the text by native speaker*

Response

As we are not native English speakers, our manuscript had been revised by a native English-speaking medical editor.