October 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12330).

Title: Increase in apoptosis by combination of metformin with silibinin in the inhibition of human colorectal cancer cells (COLO 205)

Author: Cheng-Chia Tsai, Tang-Wei Chuang, Li-Jen Chen, Ho-Shan Niu, Kun-Ming Chung, Juei-Tang Cheng, Kao-Chang Lin.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 12330

Dear Editor:

The current revision of our submission has been amended in accordance with the reviewer's comments. Changes in revised form and the point-to-point reply to comments are showed on the following page. We do hope that this revised form will meet your standards for acceptance. Thank you very much for this kind consideration.

We hope to have your final decision soon.

Respectfully,

Professor Juei-Tang Cheng, Ph.D.,F.C.P.

Corresponding author

Reviewers' Comments to Author:

Reviewer 1

1. Synergistically effect was found only in phosphorylation of AKT. Elevated PTEN expression was the same in co-treatment and silibinin alone. Increased AMPK and decreased mTOR phosphorylation was the same in co-treatment and Met alone. These authors should explain and discuss the phenomenon.

Reply:

Thank you for this kind suggestion. We added a discussion of the phenomenon you mentioned in this revision, as shown in the red text in the discussion. Thank you very much.

2. These authors still need to perform more apoptotic-related proteins such as bax, bcl-2, PARP to verify co-treatment induced both caspase-dependent and –independent pathway.

Reply:

Thank you for this comment. In this study, we aimed to provide a novel therapeutic strategy for the treatment of colon cancer, and we evaluated the possible mechanism(s) by showing the expression of Akt, PTEN, AMPK and mTOR. Additionally, the caspase-dependent and caspase–independent pathways were discussed in our original manuscript. Due to word limits, more studies at the molecular level will be performed in the future. Thus, we hope to have your kind understanding on this matter. Thank you very much.

3. The manuscript needs editing with regards to English.

Reply:

Thank you for this suggestion. We have revised this manuscript with the help of a professional editor (AJE), and the certificate was shown in the attached file. Thank you very much.

Reviewer 2

1. The major findings should be re-confirmed by the experiments using normal human colorectal epithelial cells.

Reply:

Thank you for this helpful suggestion. We reproduced the findings (Figure 1A) by using Human Colonic Epithelial Cells (HCoEpiC) according to your instructions. Thank you very much.

2. To make the finding firmer, performing inhibitor analyses are encouraged

Reply:

Thank you for this kind suggestion. In this study, we aimed to provide a novel therapeutic strategy for the treatment of colon cancer and to elucidate the possible mechanisms by determining the expression levels of Akt, PTEN, AMPK and mTOR. Due to word limits, more studies at the molecular level will be performed in the future. Thus, we hope to have your kind understanding on this matter. Thank you very much.

3. "caspase3" in page 5 line 15, page 9 line 1, page 9 line 7 and Figure 4C should be rewritten as "caspase 3" adding a space between "caspase" and "3".

Reply:

Thank you for this useful suggestion. We have corrected these errors in this revision and marked them using red- colored font. Thank you very much.

Reviewer 3

1. In the abstract, the authors should explain what Silibinin and Metformin are. They also need to explain the terms PTEN and AMPK.

Reply:

Thank you for this useful suggestion. We have added a more detailed description of what Silibinin and Metformin are, and we have explained the terms PTEN and AMPK in this revision. Thank you very much.

2. Introduction: This introduction focuses on Metformin and Silibinin and does not explain the logic of pushing apoptosis in cancer cells. Why AMPK phosphorylation and AKT phosphorylation is being studied.

Reply:

Thank you for this helpful comment. We have added additional information about metformin and silibinin- induced apoptosis in cancer cells, as shown in red text in the introduction. These revisions also explain why AMPK phosphorylation and AKT phosphorylation were studied. Thank you very much.

3. The discussion is long and should be focused on the two drugs and colon cancer.

Reply:

Based on your comment, we have modified the discussion in this revision. Thank you very much.

4. It would be helpful to have multiple other colon cancer cell lines to test than just simply one. If this is possible, additional data should be added.

Reply:

Thanks this useful comment. In this study, we aimed to introduce a novel therapeutic strategy in the treatment of colon cancer. Additional studies using multiple other colon cancer cell lines and animal models will be performed in advance. We hope to have your understanding and kindly support for this matter. Thank you very much.

5. The bibliography is extremely long, and should be shortened from 74 entries to approximately half that number.

Reply:

Thank you for this suggestion. We did decrease the size of the bibliography in this revision. Thank you very much.

We sincerely hope that this revision will meet your requirements for publication. Once again, I would like to express my warmest thanks to you for both your time and invaluable suggestions.

Thank you very much.