ANSWERING REVIEWERS

April 09, 2014

Dear Editor,

Please find enclosed the edited manuscript in World Journal of Gastroenterology (file name: NO: 9597).

Thank you very much for your e-mail concerning to our submitted manuscript (ESPS Manuscript NO: 9597). The authors are appreciated on your and reviewer's comments. Attached please find the authors responses and revised manuscript entitled "**Estradiol agonists inhibit human LoVo colorectal-cancer cell proliferation and migration through p53**". This resubmitted manuscript has been revised (marked on red). Please let me know if any further information of this manuscript needed. Peace with joy!
Sincerely yours,

Title: Estradiol agonists inhibit human LoVo colorectal-cancer cell proliferation and migration through p53

Author: Hsi-Hsien Hsu, Wei-Wen Kuo, Da-Tong Ju, Yu-Lan Yeh, Chuan-Chou Tu, Ying-Lan Tsai, Chia-Yao Shen, Sheng-Huang Chang, Chang-Hai Tsai and Chih-Yang Huang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9597

The manuscript has been improved according to the suggestions of reviewers:
Reviewers' comments:
Reviewer #1: The authors mainly focus on to explore whether estrogen or estradiol agonists inhibit human LoVo colorectal-cancer cell proliferation and migration through p53. Their findings showed that treatment with17 -estradiol and/or ER agonists in human LoVo colorectal cancer cells activated p53 and then up-regulated p21 and p27 protein levels, subsequently inhibiting the downstream target gene, cyclin D1, which regulates cell proliferation. In addition, 17 -estradiol and/or ER agonists significantly reduced the expression levels of uPA, tPA, MMP9 and β-catenin, which regulate cell metastasis.

However, there are some key concerns:

Q1: As we known, published papers have shown Estrogen and ER agonists are a potential alternative therapy in the treatment of human colorectal cancer. So, the conclusions from this manuscript are repeated.
A1: This study different cell type human LOVO cell was used. We specific found that 17 -estradiol and/or ER agonists significantly reduced the expression levels of uPA, tPA, MMP9 and β-catenin via p53 and activated p21 and p27 proteins.

Q2: P53 inhibitor could inhibit the expression of P53, but the authors did not showed the results in this manuscript, for example, in Fig. 2b and 2c, and Fig. 4b and 4C, the band of only P53i plus is missing. So, the results from these pictures do not make sense.

A2: Fig. 2c did show the p53i down-regulated the P53 protein level and Fig. 2b further showed the dose dependently inhibition of P53 levels as P53i pus E2.

Q3: Figure 6, the pictures of scratch at 0h are missing, would you please present them. In addition, would you please add the time point when the authors counted the number of migrating cells to the wound area post-wounding.

A3: We fell deeply sorry that we did not take the 0h photograph, but we did scratch out all the cells on 0h in all groups. We counted the number of migrating cells to the wound area at post-wounding 24h.

Q4: Would you please check the Fig.3a and Fig.4a, the band of α-tubulin and β-actin are very similar.

A4: Sorry, we misplaced 3a-α-tubulin. We replaced with the following bands:

Q5: As we known, many kinds of FBS contains hormone, including estrogen. Do the authors use the normal FBS or special FBS, just like the authors use phenol red-free medium in this study.

A5: We used the the normal cosmic calf serum (Hyclone, USA).Theserumis U.S.-sourced Thermo Scientific™ HyClone™ Cosmic Calf™ Serum, a superior bovine calf serum fortified with iron and naturally-derivd components for a broad spectrum of cell. We must admit that the full serum could not rule out the internal estrogen effects. However, we do believe that all the treatment groups are all in the same serum (internal estrogen) concentration.

Comments from the reviewer #2 and answers
The authors are appreciated on reviewer's comments as follows.

Reviewer #2: Comment to the Authors The manuscript by Hsi-Hsien Hsu et al. were presented for review. In this manuscript, the author investigated the inhibitory effect of estradiol agonists on human LoVo colorectal-cancer cell proliferation and migration focusing on the role of p53. Some of the data in this manuscript has a potential. There are a few concerns in this manuscript Comment

Q1: Upper images of Fig6b are not convincing. Please show more comprehensive figures.

A1: Sorry, We did try to get better image, but this is the best image we can get at this moment!

Q2: Please add the following papers in the reference. Akira Murakami. Modulation of protein quality control systems by food phytochemicals. Journal of Clinical Biochemistry and Nutrition. Vol. 52 (2013) No. 3. 215-227

A2: We already inseted in ref.33.