June 29, 2014

Dear editor:

The basic information of our manuscript as follow:

Title: Effects of oridonin-mediated hallmarks changes on inflammatory pathways in BxPC-3 cells

Author: Ru-Yi Chen, Bin Xu, Su-Feng Chen, Si-Si Chen, Ting Zhang, Jun Ren, Jian Xu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 11059

I am very happy to be received the letter of you and peer reviewers. The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated, including figures, fonts, row ledge and so on.

2. Revision has been made according to the suggestions of the reviewer whose number is 01734797.

(1) According to the results of MTT, we could find that the effect of oridonin gradually decline as the extension of the time. Therefore, MTT experiment was very important. When finished MTT, we should deal with the subsequent experiments in the shortest possible time. In this MTT test in our paper, we could select three effective drug concentrations of highest, medium and lowest for studying mechanism.

(2) We have done some research in earlier time. These researches suggested that oridonin could block the cell cycle in S phase and G2/M phase, induce cell apoptosis, and change the expression of some proteins in BxPC-3 cells. Interestingly, the function of oridonin has time and dose dependent. Meanwhile, another researches of our group have on other pancreatic cancer cell lines, such as AsPC-1 and PanC-1, for studying the anti-tumor mechanism of oridonin. We all felt so sorry for not showing the results to you as some papers have not been published yet.

(3) We were glad to learn that the changed activity of NF-kappaB pathway had still to be tested through certain functional assay, such as Electrophoresis Mobility Shift Assay (EMSA) or nuclear translocation. We are learning the related experiment and trying it.

(4) Cytokines were also tested by ELISA assay to present the extracellular level. IL-1β, IL-33, and IL-6 have statistically significant decrease in ELISA assay. In addition, we have done the western blotting experiments of IL-33, which showing the decreased intracellular

level caused by oridonin treatment. The changed expression of IL-33 was uniform both inside and outside in BxPC-3 cells. Furthermore, we have explained the significance of the intra-nuclear IL-33.

(5) We have added the discussion on the effect of p-STAT3 in this paper.

(6) The tumor cells in our lab were bought from Shanghai chinese academy sciences, which is a cells regular vendors in China. Although, BxPC-3 is known to have homozygous deletion of Smad4 gene, the western blot indicate the lower expression of Smad4 in our experiment. The expression of Smad4 was up-regulated by oridonin.

(7) The original reference 15 was cited as "we found cell apoptosis", which was indeed written by them. http://www.ncbi.nlm.nih.gov/pubmed/12767057

(8) We have edited the manuscript by native speakers of English.

3. Revision has been made according to the suggestions of the reviewer whose number is 00417178.

(1) We have discussed the points and cited related references that mentioned by reviewer.

(2) We have emphasized the potential clinical application and future perspectives arising from the results of the present study.

(3) We have explained the point better that related STAT3 in results section.

(4) We have read carefully the paper and corrected several typing and grammatical errors in the manuscript.

4. Revision has been made according to the suggestions of the reviewer whose number is 00045410.

(1) We have simplified the discussion by removing statements on background knowledge and focused on clinical relevance.

(2) We have proposed the implications of our results in planning further studies.

5. References and typesetting were corrected.

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

Sincerely yours,

Jian Xu

Jian Xu, PhD, Associate Professor,

The College of Life Science, Zhejiang Chinese Medical University

Bin Wen Road, Bin Jiang District, Hangzhou 310053, Zhejiang Province, China.

Tel: +86-571-86633001,

Fax: +86-571-86633001,

E-mail: xujian832002@163.com.