Format for ANSWERING REVIEWERS

November 5, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5462-review.doc).

Title: Oleanolic acid and ursolic acid inhibit proliferation in transformed rat hepatic oval cells

Author: Yu-Ying Han, Xiao-Wei Xue, Zheng-Ming Shi, Peng-Yan Wang, Xin-Rui Wu, Xue-Jiang Wang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5462

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

2 Revision has been made according to the suggestions of the reviewer

(1) **REVIEWER:** The authors induced malignant transformation into rat hepatic oval cells WB-F344 by low dose H2O2 treatment and evaluated the effects of OA and UA. Overall the data is convincing.

RESPONSE: We thank the Reviewer for greatly encouraging.

Addressing a few issues will improve the paper.

COMMENT: In addition to cell cycle data results from cell proliferation assays (MTT or BrdU incorporation) should be included in Fig.1.

RESPONSE: Thanks for a very good suggestion. Following the reviewer, we replenished MTT assay experiments to estimate the effects of H_2O_2 on WB-F344 cells proliferation, and the new data are shown in new Fig. 1A.

COMMENT: In page 9 it was mentioned 'an increasing nucleus to cytoplasm ration was observed, as were many mitotic cells, polykaryocytes and even tumor giant cells'. These findings need to be shown in Fig. 2.

RESPONSE: Thanks for a very good suggestion. In Fig. 2A, We have shown them with different colour arrows.

COMMENT The methodology for measuring aneuploidy is not clear and needs to be described in detail.

RESPONSE: We agree with the reviewer. Following the reviewer, we replenished the methodology for measuring aneuploidy in page 9 as following: FACS analysis was used to examine the tumorigenicity of H_2O_2 in WB-F344 cells. DNA was stained with propidium iodide to analyze cellular DNA content. The population of >4N cells represent aneuploidy cells.

COMMENT In addition to BRL cells proliferation assay needs to be performed in non-transformed WB-F344 cells upon treatment with OA and UA. This data will strengthen the paper.

RESPONSE: Thanks for a very good suggestion. Following the reviewer, we replenished MTT assay experiments to estimate the effects of OA and UA on quiescent WB-F344 cells proliferation, and the new data are shown in new Fig. 3D.

COMMENT The paper needs changes in some terminology, e.g., i. In the title: 'malignantly' should be replaced by 'transformed' ii. What is meant by 'premalignant and malignant lesion'?

RESPONSE:

i. The reviewer is absolutely right. We have replaced 'malignantly' with 'transformed'.

ii. Premalignant lesion is a morphologically altered tissue or cells in which cancer is more likely to occur than its apparently normal counterpart.

Malignant lesion is a state in which cancer has occurred.

(2) **REVIEWER:** The manuscript by Han et al. describes the effect of the phytochemicals oleanolic acid (OA) and ursolic acid (UA) on the malignant phenotype of tumor cells. They used H_2O_2 to induce proliferation and malignant transformation in the rat liver oval cell line WB-F344. The authors analyzed cell morphology and colony formation rates. Although the data presented in this manuscript is with great interest, several points should be addressed in order to improve the manuscript.

RESPONSE: We thank the reviewer for greatly encouraging.

COMMENT: My major concern is that all the conclusions obtained in this study are based on one cell line (cell line WB-F344). It will be appreciated if the obtained data were confirmed with at least with another cell line.

RESPONSE: Thanks for a very good suggestion. Presently，we are performing some other experiments using another human hepatoma cell line SMMC-7721 and HepG2, in order to further explore the mechanism of OA and UA prevention and anti-tumor.

COMMENT: Fig.2A. a global view of the figure is necessary in order to appreciate the difference between Con and H2O2 figures.

RESPONSE: Thanks for a very good suggestion. In order to appreciate the difference between Con

and H_2O_2, we enlarge some part of the picture to make the difference become visible, and we have shown this distinction using different colour arrows in Fig. 2A.

COMMENT: In Fig 2B.The cell line WB-F344 doesn't make colonies at all?

RESPONSE: It is a good question. Due to the WB-F344 cells proliferative capacity, sometimes they can produce one or two clusters, but they are not typical clone. In our experiments, we did not see the typical colonies.

COMMENT: Fig 3A, 3b, error bars are missing

RESPONSE: The reviewer is absolutely right. We added error bars in new Fig. 3A, 3B.

(3) We thank the reviewer very much for attentive review. Of all the comments, we reply comprehensively as following:

Following the reviewer, we replenished MTT assay experiments to estimate the effects of H_2O_2 on WB-F344 cells proliferation, and the new data are shown in new Fig. 1A. WB-F344 cell line has been widely used in experimental studies. Presently，we are performing some other experiments using another human hepatoma cell line SMMC-7721 and HepG2, in order to further explore the mechanism of OA and UA prevention and anti-tumor. Traditional Chinese medicine (TCM) plays an important role in maintaining human health and its benefits are gradually being recognized worldwide. The separation and extraction of effective monomer from TCM is an important direction of anti-tumor drug discovery currently. Based on a large number of chemical and pharmacological research studies, numerous bioactive compounds have been found in Chinese medicinal plants for the treatment of tumor. OA and UA as monomer drugs show potential prevention and anti-tumor properties in our experiment.

3 References and typesetting were corrected

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

Sincerely yours,

Department of pathology and physiology
Capital Medical University

You An Men Xi Tou Tiao 10
Beijing 100069, P. R. China
Tel: +86-10-83911434
Fax: +86-10-83911434
E-mail: xj-w@163.com