Format for ANSWERING REVIEWERS

May 5, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16391-review.doc).

Title: Ursodeoxycholic acid induces apoptosis in hepatocarcinoma xenografts in mice

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16391

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

1 The format has been updated according to the policies of the journal.

2 We have addressed the comments of the first reviewer as follows:

Major comments:

(1) We have already published work comparing the effects of UDCA on human hepatoma cell lines HepG2 and BEL7402 and a normal human hepatic cell line L-02 *in vitro*. We found that UDCA selectively inhibits proliferation and induces apoptosis in HepG2 and BEL7402 cell lines, but does not induce apoptosis in the normal L-02 cell line. BEL7402 cells were derived from a surgical specimen obtained from a 53-year-old male patient with hepatocarcinoma in 1974. The cell line is highly tumorigenic in nude mice, and some histological characteristics of primary human liver cancer are conserved in the xenografts in mice. Furthermore, the present study is performed *in vivo* and thus represents a natural progression from our previous study, which explored the potential of UDCA treatment on

BEL7402 *in vitro*.

(2) We have revised Figure 1 based on the suggestions of the reviewer and the editor.

(3) We have added the following information to the Materials and Methods section and the Results section.

Body weights of animals in each group were measured before initiation of the experiment and after 21 days. Tumor growth was measured once each week over the 21 days, and tumor volume (V) was calculated as $V = (L \times W^2) \times 0.52$, where L was the length and W was the width of a xenograft.

The mean body weight for all groups on day 0 before implantation was 17.8 ± 1.8 g. At day 21, body weights generally had decreased as xenografts developed with the most dramatic decrease in untreated controls. The mean body weight was 14.5 ± 1.5 g, 15.7 ± 1.6 g, 16.7 ± 1.7 g and 17.6 ± 1.8 g for controls and the UDCA groups at 30, 50 and 70 mg/kg/day, respectively. Statistical analysis demonstrated that body weight was significantly different between the treatment groups and controls (30 mg/kg/day, $P < 0.05$; 50 mg/kg/day, $P < 0.05$; 70 mg/kg/day, $P < 0.01$).

(4) We hope that the figures can be altered by the editor in order to save space.

(5) Our manuscript has been professionally edited by AmEditor Inc.

Minor comments:

1. We have revised the Materials and Methods section according to the comments.

2. The BEL7402 cell line was derived from a surgical specimen obtained from a 53-year-old male patient with hepatocarcinoma in 1974. For these experiments, the BEL7402 cell line was obtained from the Shanghai Institute of Cell Biology of the Chinese Academy of Sciences (Shanghai, China).

3. Results

a. We have revised the first paragraph.

b. Investigators (Tschimer A, von Haehling S, Palus S, Doehner W, Anker SD, Springer J. Ursodeoxycholic acid treatment in a rat model of cancer cachexia. J Cachexia Sarcopenia Muscle. 2012, 3(1):31-36) have previously used two different concentrations of UDCA (25 or 100 mg/kg/day) to study the effects of UDCA in rats. Based on their results, we used two different concentrations of UDCA (20 or 90 mg/kg/day) to perform preliminary tests. We found that UDCA at a dose of 20 mg/kg/day had no obvious effect on BALB/c nude mice bearing s.c. BEL7402 xenografts and that UDCA at a dose of 90 mg/kg/day caused diarrhea. Based on the combined results, we used 30, 50 and 70

mg/kg/day UDCA.

c. We have revised the repetition of the dose unit according to the comments.

3 We have addressed the comments from the second reviewer as follows:

(1) We have already published work comparing the effects of UDCA on human hepatoma cell lines HepG2 and BEL7402 and a normal human hepatic cell line L-02 *in vitro*. We found that UDCA selectively inhibits proliferation and induces apoptosis in HepG2 and BEL7402 cell lines, but does not induce apoptosis in the normal L-02 cell line. BEL7402 cells were derived from a surgical specimen obtained from a 53-year-old male patient with hepatocarcinoma in 1974. The cell line is highly tumorigenic in nude mice, and some histological characteristics of primary human liver cancer are conserved in the xenografts in mice. Furthermore, the present study is performed *in vivo* and thus represents a natural progression from our previous study, which explored the potential of UDCA treatment on BEL7402 *in vitro*.

(2) Investigators (Tschimer A, von Haehling S, Palus S, Doehner W, Anker SD, Springer J. Ursodeoxycholic acid treatment in a rat model of cancer cachexia. J Cachexia Sarcopenia Muscle. 2012, 3(1):31-36) have previously used two different concentrations of UDCA (25 or 100 mg/kg/day) to study the effects of UDCA in rats. Based on their results, we used two different concentrations of UDCA (20 or 90 mg/kg/day) to perform preliminary tests. We found that UDCA at a dose of 20 mg/kg/day had no obvious effect on BALB/c nude mice bearing s.c. BEL7402 xenografts and that UDCA at a dose of 90 mg/kg/day caused diarrhea. Based on the combined results, we used doses of 30, 50 and 70 mg/kg/day UDCA.

(3) We have added more references published in the last 5 years.

(4) We have revised Figure 1 and Figure 3 as suggested by the reviewer.

4 The references and typesetting have been corrected according to the policies of the journal.

Thank you again for the opportunity to publish our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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