

Format for ANSWERING REVIEWERS

December 12, 2015

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 22801-Edited.doc).

Title: Human cytomegalovirus encoded US28 found in colorectal cancer may act as a tumor promoter

Author: Zhen-Zhai Cai, Jian-Gang Xu, Yu-Hui Zhou, Ji-Hang Zheng, Ke-Zhi Lin, Shu-zhi Zheng, Meng-Si Ye, Yun He, Chang-Bao Liu, Zhan-Xiong Xue

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 22801

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 one

1. 1) RESULTS (pg.11, lanes 22-29): paragraph "US28 expression in colorectal carcinoma and adjacent noncancerous colorectal tissue": Are the Authors sure that the US28 expression is negative in cancer tissues from patients negative for HCMV infection? In other words: can the authors exclude that the positivity observed is not due to some cross-reaction of the US28-specific antibody used with some other protein over-expressed in cancer tissue (for example, human chemokine receptors)? A control performed on samples from HCMV negative patients should be added to confirm the specificity of the antibody used.

Answer: Thank you very much for your suggestion. We had performed immunohistochemical analysis using US28 antibody in 16 cases of HCMV negative CRC specimens. US28 expression were all negative in all HCMV negative CRC specimens. Added information are as following (page 6, lanes 17 in the "MATERIALS AND METHODS") : "Sixteen additional CRC cases not infected with HCMV were used as controls.", and (page 10, lanes 15-16 in the "RESULTS") : "All cases of colorectal carcinoma

without HCMV infection exhibited negative expression (Figure 1D).”,and (page26,lanes 6-7):” Negative US28 protein expression was observed in all HCMV-negative colorectal carcinoma(D)”

2. 2) RESULTS (pg.12, lanes 8-9): paragraph “Relationship between US28 expression and age, sex, tumor site, histological grade, metastasis, Dukes’ stage and prognosis”: The Authors wrote: “High US28 expression was associated with metastasis, advanced stage and poor patient prognosis”... In Table 1 it is shown that in the advanced stages (C-D) the % of highly positive tissues is about 27.3% (15 out of 55 total at C-D stage) vs about 52% of highly positive cancer in the early stages (25 out of 48 total at A-B stage)... this means that at advanced stage the % of highly positive tissues decrease and not increase...

Answer: Thank you very much for your suggestion. I’m sorry! We got the figure wrong when filling in the form. In fact, the data of dukes stage C-D should be swapped with the data of dukes stage A-B. We have corrected it in table 1. Please see the page 25 table 1.

3. 3) DISCUSSION (pg14, lanes 8-9): “For the colorectal tumor patients with follow-up data, our results indicated that high US28 expression was correlated with histological grade , metastasis and Dukes’ stage....” Based on the data reported in Table 1, it seems that high US28 expression is inversely correlated with the histological grade and Dukes’ stage, while it is directly correlated with metastasis. The Author should better clarify and discuss this point.

Answer: Thank you very much for your suggestion. I’m soory! We make a error expression because of poor English. We have corrected it. Added information are as following (Page12, lanes 13-16 in the “**DISCUSSION**”):” Based on CRC patients with follow-up data, our results demonstrated that high US28 expression is inversely correlated with histological grade and Dukes’ stage, and directly correlated with metastasis, which indicated US28 may be involved in the progression of CRC.”

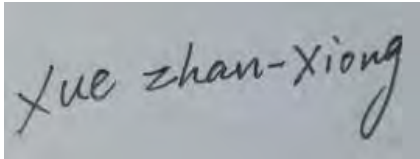
4. Minor points: 4) In some points of the manuscript the English is not fluent or with grammar errors, and should be revised with the help of a native English auditor. An example (pg.13 lanes 9-14): “As shown in Figure 7, compared to the nontransfected control group and the negative control, transfection with pCMV6-entry-US28 resulted in a 82.9% (P=0.001) and 93.9% (P=0.000) increase respectively, in the invasion of cells into the transwell plate chamber.” English is not fluent: it should be better: “As shown in Figure 7, transfection with pCMV6-entry-US28 resulted in a 82.9% (P=0.001) and 93.9% (P=0.000) increase of cells invasion ability, compared to

Answer: Thank you very much for your suggestion. The paper had been modified by the company of American Journal Experts. It has been revised with the help of a native

English editor.

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

Sincerely yours,

A handwritten signature in black ink on a light gray background. The signature reads "Xue zhan-xiong" in a cursive, slightly slanted script.

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