Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4530-review.doc).

Title: Curcumin represents enhanced cytotoxicity to colorectal cancer cells with PTEN deficiency

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Name of Journal: *World Journal of Gastroenterology*

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

1 Format has been updated
Response: Yes.

2 Revision has been made according to the suggestions of the reviewer
Comments from Reviewer 1:
(1) The manuscript attempted to demonstrate that the functional status of the PTEN gene effected on curcumin cytotoxicity for CRC cells, but the authors had not described the mechanism of curcumin cytotoxicity. Why they did not choose the other tumor suppressor gene, for example, P53 gene, to investigate the relationship between the gene function and curcumin cytotoxicit?
Response: Following the reviewer's suggestion, we investigated the relationship between p53 status and the curcumin cytotoxicity. We found that p53 deficiency did not affect the cytotoxicity of curcumin toward HCT116 cells. This data was added to Figure 2 (F). The figure legend and text were modified accordingly.
Please see the "Results" section, paragraph 2 ("***Enhanced cytotoxicity of curcumin toward PTEN-deficient cancer cells***"):
"Next, we determined whether curcumin also shows increased cytotoxicity toward HCT116 cells deficient in p53. However, we found that disruption of p53 had no effect on the sensitivity of HCT116 to curcumin (Figure 2F)."

(2) The authors developed the isogenic set of human CRC cells that differ only in their PTEN status, PTEN+/+, PTEN+/-and PTEN-/-, but they did not describe the characteristic differences of these cells that they developed.
Response: To further characterize the PTEN positive and PTEN null cells as the reviewer suggested, we analyzed the morphology of these cell lines. We found that PTEN positive and PTEN null cells had a similar morphology. This data was added to Figure 1 (E). The figure legend and text were modified accordingly.

Please see the “Results” section, paragraph 1 (“***Targeted deletion of PTEN in colorectal cancer cells***”):
“*PTEN*$^{-/-}$ cells displayed a similar morphology to the parental *PTEN*$^{+/+}$ cell line (Figure 1E).”

(3) The mechanism elaborated unclearly. The results in this manuscript indicated that PTEN deficiency could change the curcumin-induced cell cycle arrest pattern and resulte in an increased sensitivity of HCT116 cells to curcumin. Although the authors showed us the different expression levels of P21, Cyclin D1, Cyclin B1, Cdc2 and p-Akt between the PTEN+/+and PTEN-/- cells. However, these are not sufficient for the explanation of curcumin cytotoxicity differences in these cells
Response: A recent report published in Science suggested that PTEN functions in DNA repair and thus controls sensitivity to genotoxic stress (*Science*, 2013, 341: 395-9.). DNA damage is one of the molecular events associated with cell cycle arrest. To further explore the mechanism of curcumin cytotoxicity to PTEN null cells, we investigated the expression levels of p27, another G0/G1 phase regulator as well. However, we found that there was no difference in the p27 levels between PTEN positive and PTEN null cells after curcumin treatment. This data was added into Figure 5 (B). Further studies are needed to fully understand the detailed mechanism.
Please see the "Results" section, paragraph 4 ("***PTEN deficiency results in altered curcumin-induced cell cycle arrest***"):
"p27 is another known regulator of G0/G1 phase; however we observed no difference in its expression levels between the *PTEN*$^{+/+}$ and *PTEN*$^{-/-}$ cells following curcumin exposure (Figure 5B)."

(4) Insufficiency citation for the research literatur. The results in ths manuscript showed that curcumin exposure led to a marked G2/M phase cell cycle arrest in the cells with wide-type PTEN and a significant G0/G1 phase arrest in PTEN-/- cells. But the authors had not cited this publication that showed “curcumin can induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma” (Biochem Pharmacol. 2005; 70:700-13).
Response: As the reviewer suggested, we added the reference of “Curcumin (diferuloylmethane) inhibits constitutive NF-kappaB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma” (Biochem Pharmcol 2005; 70:700-13) to the “Discussion”.

(5) The manuscript is full of grammatical errors and awkward sentences. It should be fully revised for the English.
Response: Following the reviewer’s suggestion, we have fully revised our manuscript for correct English usage, style and spelling.

Comments from Reviewer 2:
(1) The manuscript is consistent and well structured, but English style and spelling (including the title) should be reworked.
Response: As suggested by Reviewer 2, we have revised our manuscript for English

usage, style, and spelling. Also see our response to Comment 5 from Reviewer 1.

(2) In the abstract, the authors should mention in the results that PTEN knockout did not affect the cytotoxicity of CPT-11, 5-FU, oxaliplatin, or DHA.
Response: A Following the reviewer's suggestion, we added our finding that PTEN deficiency did not affect the cytotoxicity of CPT-11, 5-FU, oxaliplatin, or DHA to the "Abstract":
"Using this set of cell lines, we found that disruption of the *PTEN* gene had no effect on the sensitivity of CRC cells to 5-FU, CPT-11, DHA or OXA, whereas *PTEN* disruption increased the sensitivity of CRC cells to curcumin"

3 References and typesetting were corrected
Response: Yes.

Sincerely yours,
Xincheng Lu