The World Journal of Gastroenterology Editorial Team

28th of April 2013

Dear Editor,

Thank you very much for giving us the opportunity to revise our manuscript. We appreciate very much the Reviewers' valuable comments, and we have reviewed the comments by the reviewers, found them to be very clear and insightful, and have revised the manuscript accordingly. We detail our point-by-point responses in the following pages. We hope that our revised manuscript will be accepted by the World Journal of Gastroenterology editorial team. If you still have any questions to our responses, don't hesitate to inform us, and we will answer them as soon as possible. **The revised parts are highlighted in red font in the revised manuscript.** Please find enclosed the edited manuscript containing an electronic copy of the full-text manuscript in Word format (file name: wjg-2013-2874-Review.doc).

Title: Reversal of multidrug resistance in gastric cancer cells by CDX2 downregulation

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Manuscript No: Ms.wjg/2013/2874

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：

Reviewer: 00505502

The comments of the reviewer 1 are extremely valuable.

Comments (1): *The authors should examine whether the CDX2 can regulate the resitance of CDDP using by SGC7901/DDP and SGC7901 normal cell line.*

Responses 1: This is a very good point and we thanks for reviewer's comments. First, the purpose of our study is to investigate down-regulation of CDX2 affect drug resistant cells, but SGC7901 normal cell line can't tolerate CDDP in experimental concentration, SGC7901 normal cell line will die in the environment with CDDP, Which is demonstrated by our preliminary experiments. Thus, this will not have much meaning to use SGC7901 normal cell line to study the cell drug-resistance. Second, as we known, many published articles about drug resistance reversal is not using normal cell line as negative control group. Such as：**1.** Hong L, Zhao Y, Wang J, Han Y, Guo W, Jin H, Zhai H, Bai F, Zhang X, Qiao T, Chen Z, Fan D. Reversal of multidrug resistance of adriamycin-resistant gastric adenocarcinoma cells through the up-regulation of DARPP-32. Dig Dis Sci 2008; 53: 101-107; **2.** Hong L, Qiao T, Han Y, Han S, Zhang X, Lin T, Gao J, Zhao P, Chen Z, Fan D. ZNRD1 mediates resistance of gastric cancer cells to methotrexate by regulation of IMPDH2 and Bcl-2. Biochem Cell Biol 2006; 84: 199-206; **3.** Han Z, Hong L, Han Y, Wu K, Han S, Shen H, Li C, Yao L, Qiao T, Fan D. Phospho Akt mediates multidrug resistance of gastric cancer cells through regulation of P-gp,Bcl-2 and Bax. J Exp Clin Cancer Res 2007; 26: 261-268; **4.** Hong L, Wang J, Han Y, Zhao Y, Gao J, Wang J, Han Y, Zhang X, Yan L, Zhou X, Qiao T, Chen Z, Fan D. Reversal of multidrug resistance of vincristine-resistant gastric adenocarcinoma cells through up-regulation of DARPP-32. Cell Biol Int 2007; 31: 2010-1015; **5.** Hong L, Wang J, Zhao Y, Han Z, Zhou X, Guo W, Zhang X, Jin H, Wu K, Ding J, Fan D. DARPP-32 mediates multidrug resistance of gastric cancer through regulation of P-gp and ZNRD1. Cancer Invest 2007; 25: 699-705).**Therefore,** we do not choose SGC7901 normal cell

line as negative control group.

Comments (2): *The authors measured IC 50 values of SGC7901/DDP cells exposed to some cytotoxic agents such as, cisplatin, adriamycin, and fluorouracil. The authors examined the effect of adriamaycin in this study. Cisplatin or fluorouracil is key drug for gastric cancer in the clinical setting. Therefore, the authors should show the date about these agents and have more discussion about it.*

Responses 2: The reviewer is absolutely correct, a table has been added to show the data about the IC50 of Cisplatin , fluorouracil and DDP（see pages 21）. In addition, we do more discussion about these agents in discussion section, If you still think some content should be added to discussion section, don't hesitate to inform us, and we will add them as soon as possible.（see pages 14）

Comments (3): *The expression of CDX2 by Western blot is poor. The authors should carry the more clearly picutures.*

Responses 3: This is a valid point by the reviewer. We have removed the figures (Figure 1B), and added a clearly picuture. If you still think some figures should be replaced, don't hesitate to inform us, and we will change them as soon as possible. （see pages 23）

Reviewer: 02440197

The comments of the reviewer 2 are extremely inspiring.

Comments (1)*: In my opinion, the manuscript needs some language modifications and can be accepted for publication in World Journal of Gastroenterology.*

Responses 1: The reviewer is absolutely correct that we have submitted our

manuscript to professional English language editing company (Jing-Yun Ma Editorial Office) to English proof-read our manuscript. Please see the editing certificate which is from English language editing company. It can verify that our final manuscript has been English proof-read by that company.

Reviewer: 00502831

The comments of the reviewer 3 are extremely valuable.

***Comments (1)**: I How about the expression of apotosis related gene auch as p53, bax, bcl-2, and caspase3 et al.?*

Responses 1: To investigate the mechanism by which Cdx2 siRNA induces apoptosis in SGC/7901cells, we detected expression levels of several apoptotic family members including caspase-9, caspase-3, p53, bax, bcl-2, Survivin, and c-Myc by semi-quantitative RT-PCR and Western blot analysis. **We found that down-regulation of CDX2 induced the expression of c-Myc, Survivin and Cyclin D1 in SGC7901/DDP cells showed a significant decrease** (see page 11), we also studied the expression of apotosis related gene (caspase-9, caspase-3, p53, bax and bcl-2), but the protein expression differences have no statistical significance, so we don't list them. Therefore, in the present study, down-regulation of CDX2 may decrease c-Myc and Survivin expression directly or indirectly, leading to induce apoptosis in SGC/7901cells, but it does not affect the other apotosis related gene (caspase-9, caspase-3, p53, bax, bcl-2) expression. **Thus, we suppose that down-regulation of CDX2 induces reversal effect of multidrug resistance in gastric cancer cells via the c-Myc/Survivin pathway.**

***Comments (2)**: The authors had better to show the expression of CDX2 protein or mRNA on the tumor, immunohistochemically or in situ hybridization in Fig.4A.*

Responses 2: This is a very good point and we thanks for reviewer's

comments. We have *showed the expression of CDX2 protein and mRNA on the tumor* by using Semi-quantitative RT-PCR and Western blot. If you still think some pictures should be added, don't hesitate to inform us, and we will add them as soon as possible.（see pages 26）

Reviewer: 02441494

The comments of the reviewer 4 are extremely valuable.

Comments (1)*: The language of the manuscript is poor.*

Responses 1: We feel so sorry about our poor English writing and thanks for reviewer's suggestion. We have submitted our manuscript to professional English language editing company (Jing-Yun Ma Editorial Office) to English proof-read our manuscript. Please see the editing certificate which is from English language editing company. It can verify that our final manuscript has been English proof-read by that company.

Comments (2)*: Line 45-48: The meaning of the sentence is confusing.*

Responses 2: The reviewer is right that we were sloppy on our way of presentation which causes confusion to the readers, we have submitted our manuscript to professional English language editing company, and wrote these sentences. （see pages 3）

Comments (3)*: Line 117-126: MTT is a very old technique for research. It would be more accurate and time-saving to use ATP-TCA or CD-DST for scientific research.*

Responses 3: We agree with the reviewer on this point and will choose the new method of detecting cells survive and grow to make further study of Cdx2 functions in the future. The reasons why we choose the MTT method are as follows: First, MTT is a classic method of detecting cells survive and grow, we can see it used in many published research (**1.** He G, Lei W, Wang S, Xiao R,

Guo K, Xia Y, Zhou X, Zhang K, Liu X, Wang Y. Overexpression of tumor suppressor TSLC1 by a survivin-regulated oncolytic adenovirus significantly inhibits hepatocellularcarcinoma growth. J Cancer Res Clin Oncol 2012; 138: 657-670; **2.** Radetzki S, Köhne CH, von Haefen C, Gillissen B, Sturm I, Dörken B, Daniel PT. The apoptosis promoting Bcl-2 homologues Bak and Nbk/Bik overcome drug resistance in Mdr-1-negative and Mdr-1-overexpressing breast cancercell lines. Oncogene 2002; 21: 227-238; **3.** Yang C, Cai J, Wang Q, Tang H, Cao J, Wu L, Wang Z. Epigenetic silencing of miR-130b in ovarian cancer promotes the development of multidrug resistance by targeting colony-stimulating factor 1. Gynecol Oncol 2012; 124: 325-334) Second, in our Preliminary experiments, the data form MTT method is more stable and reliable than CCK-8 and CD-DST methods in this SGC7901/DDP cell line. **Therefore, we choose the MTT method.**

***Comments (4)**: Line 167: OD280 is an accurate and widely accepted technique, however, Lowery assay is tedious and needs larger amount of sample.*

Responses 4: The reviewer is right that we were sloppy on our way of presentation which causes confusion to the readers. We choose the Western blotting analysis to detect the expression levels of Survivin, Cyclin D1, and c-Myc protein. Lowery assay is only one step of Western blotting analysis, but our sloppy presentation causes confusion to the reviewer, so we Modify the description of Western blotting analysis (see pages 8).

***Comments (5)**: Discussion should focus on what you have got in your experiment, not just review.*

Responses 5: This is a very good point and we thanks for reviewer's comments. We have revised the discussion section, in the new discussion, we focus on what we have got in our experiment and made them in detail (see pages 12-14), and the composition of review has been condensed into a

paragraph, If you still think some changes should be made, don't hesitate to inform us, and we will do them as soon as possible.

Reviewer: 00070288

The comments of the reviewer 5 are extremely encouraging.

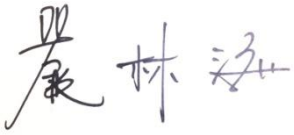
Comments (1)*: The manuscript is now a good piece of work. I have found one minor mistake: Result 1"7901/DDP" should be "SGC7901/DDP". The Submission has been greatly improved and is worthy of publication.*

Responses 1: We feel so sorry about this very Low-level mistakes and thanks for reviewer's suggestion. We will strictly check, not appear this kind of mistake again.

3. References and typesetting were corrected

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

Sincerely yours,



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