Author's response to reviews

Title: Methylation of WTH3, a Possible Drug Resistant Gene, Inhibits p53 Regulated Expression

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Author's response to reviews: see over
Dear Robin Cassady-Cain,

Thank you for your e-mail and the second reviewers’ comments about our manuscript entitled, “Methylation of WTH3, a Possible Drug Resistant Gene, Inhibits p53 Regulated Expression” (MS 1388503408194259). We are encouraged by the positive comments and the possibility of our article being published in your journal.

Based on your instructions, we will address the questions and suggestions posed by each reviewer:

Reviewer: Volodymyr Tryndyak (in italic font)

I would like to thank the author for their answers to my comments.
I have no other major comments, but my second question was about the actual value IC50 of Dox for MCF/7AdrR and MCF7/WT cell lines, not the definition of IC50.

Response (in regular font):

We are sorry for misunderstanding the reviewer’s question about the IC50 value of Dox for MCF/7AdrR and MCF7/WT cell lines. The actual IC50 value of Dox for MCF/7AdrR and MCF7/WT cell lines was about 975 nM and 1.25 nM, which were published in our previous publication (Shan, J, Mason, JM, Yuan, L, et al. Rab6c, a new member of the rab gene family, is involved in drug resistance in MCF7/AdrR cells. Gene 2000; 257:67-75). This paper was cited as reference #10 in the manuscript. We added this information in “Cell lines and Treatment” in the “Materials and Methods” section.

Also, if it is possible, please provide standard deviation bars on Figures 1, 3 and 4 to show statistical analysis. These recommendations the author can choose to ignore.
In general, the authors have answered my points sufficiently well to allow their manuscript to be published.

Our each experiment was repeated more than three times and similar results were obtained. The highs of the columns in figure 1B, 3B and 4B reflected the exact quantities of the PCR products in 1A, 3A and 4A, respectively. Therefore, as per the reviewer’s comments, we choose to ignore this particular recommendation.

Reviewer: Pearly Yan (in italic font)

1) Gel based expression studies should really be used as a tool for quick initial checks. The authors should provide quantitative data in the manuscript to benefit the readers.
Response (in regular font):

We agree with the reviewer’s comment. In fact, to measure each gene product of interest, we performed SQRT-PCR assays more than three times. We added this information in the “SQRT-PCR” portion of the “Materials and Methods” section.

We did provide quantitative data for WTH3 transcripts in WTH3 knockdown assays. In the last sentence on page 8 of the original manuscript, we wrote: “The results showed that WTH3 transcripts in the cells infected with pSIEN-RetroQWTH3 (293/WTH3RNAi-P) were about 2 times lower than that in the control cells containing the empty vector (Fig. 1A, 1B). In the revised manuscript, following the word “result”, we added: “obtained from the three individual measurements”.

We did not provide quantitative data for MDR1 transcripts in WTH3 knockdown assays. This was because MDR1 transcripts in the negative control, 293/WTH3RNAi-P, were undetectable. Therefore, the sentence, “…the results showed that the MDR1 gene was re-activated in the 293/WTH3RNAi-P, 293/WTH3RNAi-2, -3 and -6 cells, while MDR1 transcripts were undetectable in the corresponding control cells (Fig. 3A, 3B)” in the first paragraph, of page 9 in the original manuscript, remained unchanged.

2) 5-aza is a well used compound; however, it is also known that it does not induce demethylation all over the genome. Therefore, it is pertinent to evaluate the methylation status of WTH3 gene promoter before and after 5-aza treatment to claim that MDR is a result of promoter demethylation of WTH3 gene.

We agree with the reviewer. As a result, we no longer claim that 5-aza demethylated the WTH3 gene promoter. Therefore, we altered our original conclusions derived from the 5-aza assays.

We revised the first sentence of the last paragraph of page 9 in the original manuscript from, “DNA methylation of the WTH3 promoter interfered with gene expression activated by p53” to “5-aza treatment promoted p53’s positive impact on WTH3 expression.”

We also revised “…and then treated the transfectants with 5-aza to see if the p53 transgene preferentially activated the demethylated promoter, but not the methylated one” in the first paragraph, of page 10 in the original manuscript to “…and then treated the transfectants with 5-aza to see if the p53 transgene preferentially activated WTH3 gene expression, but not in the untreated cells.

In addition, we revised “We found that the p53 transgene’s positive effect on the WTH3 gene promoter of the cells treated with 5-aza was about 2 times stronger than…It was observed that the p53 transgene and 5-aza positively affected the WTH3 promoter of the cells compared to the untreated cells…”, in first paragraph, of page 10 in the original manuscript to:

“We found that the p53 transgene’s positive effect on WTH3 gene expression of the cells treated with 5-aza was about 2 times stronger than…It was observed that the p53 transgene and 5-aza positively affected WTH3 expression of the cells compared to the untreated cells…”
Hopefully, the merit of the revised manuscript will meet your scientific requirements and be published in your journal.

Thank you for your consideration of the enclosed manuscript.

Sincerely,

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