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 three 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**

1. What is 5-aza? Provide the full name for this abbreviation.

   **Answer:** 5-aza is the abbreviation for 5-aza-2’-deoxycytidine. We added its full name in introduction section when it was first time appeared (page 4, last paragraph).

2. In “Materials and Methods” please indicate, what is the IC50 of Dox for the MCF7/AdrR and MCF7/WT cell lines?

   **Answer:** IC50 values represent Dox concentrations that cause 50% cell death. We added this information in “MTT Assay” in the “Materials and Methods” section.

3. In addition to the successful WTH3 knockdown shown by SQRT-PCR (Figure 3), I would recommend that the authors do Western blot analysis or cytochemistry for the MDR1 protein on HEK293 and MCF7/AdrR cell lines.

   **Answer:** We agree with the reviewer’s suggestion. However, due to the close similarity between WTH3 and Rab6c gene products, we encountered cross interaction with those two proteins when Western blot was performed, therefore, we did SQRT-PCR.

4. In literature, there are a lot of data regarding promoter hypomethylation of genes that led to gene over expression and development of multi-drug resistance in the tumor or decreased apoptosis during cancer treatment. In view of this, the authors must be cautious in their recommendation of restoring or increasing genes’ activity as a strategy for eradicating MDR encountered during cancer chemotherapy by introducing DNA demethylation reagents.

   **Answer:** We agree with the reviewer. MDR development during cancer chemotherapy is a very complex biological process. Using DNA demethylation reagents to increase the genes’ activity (such as WTH3) could be one recommendation, however, the mechanisms for such a strategy still remain to be established.
Reviewer: Edward Ratovitski

Comments 1 to 9 were very positive. Thank you.

I believe this is a solid work with important implications for cancer biology and pharmacology and I am suggesting the acceptance of this manuscript with minor essential revisions, which would include the correction of Fig.1A (upper panel should be redone to provide publication quality and avoiding vertical bar after sample”239-V”).

Answer: We erased the vertical bar in the Fig. 1A.

Figure 2 requires standard deviation bars.

Answer: We added standard deviation bars in Figure 2.

Reviewer: Pearlly Yan

Areas that need clarifications:

1. The gene expression studies are all gel-based and the data expressed in relative units. Even though the authors suggested that the WTH3 knockdown is successful (WTH3 expression at 2 or more times below the original level), readers would be able to evaluate this effect if the original level is presented.

Answer: The original level of WTH3 is presented in the first two lanes in Figure 1A and 1B. The first lane in both A and B represent the level before the gene is knocked down, while the second lanes are also considered the gene’s original expression level although the empty vector was introduced into the host cells.

2. Even though the authors presented data on the effect of 5-aza treatment to various constructs, it is important to show that the demethylation actually occurs in the WTH3 promoter and that it is not a downstream effort. The authors should modulate their claim in the Discussion section (page 12, final paragraph) regarding the demethylation of WTH3 gene promoter can "eradicate" MDR.

Answer: 5-aza, which inhibits methylation in the entire genome, is a popular reagent and widely utilized for DNA methylation studies. Based on the reviewer’s suggestion, we replaced the word “eradicating” to “easing” in the Discussion section (page 13, final paragraph).

3. It is not justifiable to introduce acronyms that appear only once in the manuscript (page 12, paragraph 2: EMFs and MBPs).

Answer: Actually, it is justifiable to introduce acronyms, MBPs and EMFs, since in the
same sentence they are mentioned again.

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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