Author's response to reviews

Title: Effect of sulfasalazine on human neuroblastoma: analysis of sepiapterin reductase (SPR) as a new therapeutic target

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Research article
Effect of sulfasalazine on human neuroblastoma: analysis of sepiapterin reductase (SPR) as a new therapeutic target
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BMC Cancer (Section: Experimental therapeutics and drug development)

Dear Editor,
We have responded point-by-point to the reviewers’ questions (see below) and all changes in the manuscript have been highlighted by red colored text. We also changed Figure 3 and Figure 4 in order to incorporate the proposed reviewer recommendations. Two references have been added for further clarification (Ref 39, 40) and one reference has been updated (Ref 26, previously AACR abstract, now paper in PloS One, in press). We trust that the revised manuscript is now acceptable for publication in BMC Cancer and look forward to your response.

Reviewer 1: Jianhua Yang

Reviewer's report:
In the submitted manuscript by Lisette P.Yco et al., the authors investigated the function and clinical relevance of sepiapterin reductase (SPR) in human neuroblastoma (NB). SPR expression was showed to correlate with unfavorable NB characteristics and therapeutic treatment by blocking SPR with sulfasalazine (SSZ) led to potent anti-proliferation activity in vitro. In addition, the manuscript identified sulfasalazine in combination with DFMO as a novel therapy for NB patients.

Although this study is interesting with carefully designed experiments, some points should be clarified.

We thank the reviewer for the kind remarks, and have addressed the issues below.

Major points:
1. In figure 3, Manuscript showed that “SPR and ODC (2 log) expression values for each sample are visualized with red circles and black rectangles”, but there is no red circles and black rectangles in figure.

   The color codes for SPR and ODC1 expression values in Figure 3 were reversed. We have corrected this in a new Figure 3. The legend for Figure 3 is now correct. We apologize for the error.

   In further explanation of Figure 3: The figure shows the mRNA expression levels for SPR and ODC1 for all samples of the Kocak-649 dataset. From left to right, the 649 tumor samples are ordered by their SPR expression. The actual expression values are shown, for each sample, by a red circle (for SPR, values on left y-axis), and a black rectangle (for ODC1, values on right y-axis). We readily admit the circles and rectangles are small, but increased size did not lead to increased clarity of the figure because of the high number of samples. We propose that the different colors, as well as the steadily rising SPR expression values from left to right enable the reader to distinguish between the SPR and ODC1 expression values for each tumor.

   In addition, even though the p value is very significant, but the R value is only 0.225, which showed very weak correlation between SPR and ODC. Therefore, the rationale of targeting SPR and ODC together is not well established.

   The reviewer is right in pointing this out. Within a dataset this size, an association of 0.225 may appear weak. However, as expressed by the P value, the possibility of finding such a correlation by chance is extremely small. In addition, we found a similar correlation (R = 0.289, P = 6.2 • 10^{-3}) in the Versteeg-88 NB dataset in our earlier study [32]. We therefore feel strengthened in our argument that the correlation found may be meaningful. We have altered lines 116-119 to better describe this. Still, we agree with the reviewer that “offers the ratio for investigating” might be worded a bit strong, and have altered lines 122-124 accordingly.
2. For drug synergy, the synergism means that combing two drugs together can enhance or magnify the effects of these drugs. It doesn’t depend on drug concentration. Otherwise, how to explain that using same drugs with different concentrations can get different effects (symmetry or antagonism)?

Synergism and enhancement are two different concepts for combinational studies. Synergy is “mutual” while enhancement is “one-sided.” Dr. Ting-Chao Chou described synergism as two drugs, each one having an effect, and when combined may produce a synergistic, additive, or antagonistic effect. On the other hand, if drug A has an effect and drug B does not, but when combined, result in a better effect than drug A alone, it is referred to as enhancement, and not synergism (see Dr. Chou’s references 1,2). Here we showed that each drug (SSZ and DFMO) has an inhibitory effect on NB cells and when combined have synergistic, additive, and antagonist effects, dependent on concentrations. Dr. Chou’s references have been added to the manuscript (line 261; Refs 39, 40).

The interaction between drugs or biological agents is a very important factor in therapeutics-, toxicity-, and physiology studies. The interaction of two drugs is simply described as synergistic (working together) or antagonism (working against each other). Therefore, it was very important to examine the interaction of SSZ and DFMO in NB cells before moving forward to pre-clinical animal models. In order to determine the the type of interaction of SSZ and DFMO, we used the well-established isobologram and combinational index analysis approach (as described in the Methods section and references 1-3). Briefly, we first determined the IC50 values (single agent effect) of DFMO and SSZ in NB cells. We then used the IC50 of each inhibitor to design SSZ-DFMO concentration ratios to evaluate which dose combinations are synergistic, additive, or antagonistic. This approach is well established and described in the literature (references 1-3).

3. Fig.4 should include the link of significant difference and Descriptions of statistical methods.

The significant differences (p values) and a description of statistical methods have been included in the revised Figure 4 and the Methods section in the text.

Minor points:
1. Line-115 should be “P values < 0.05"
We have corrected this in the revised manuscript.

Reviewer: Mi Heon Ryu

Reviewer's report:
This is an interesting manuscript reporting the effect of sulfasalazine on human neuroblastoma: analysis of sepiapterin reductase (SPR) and a possibility of new therapeutic target. The authors investigated the expression of SPR in NB tumors, the inhibition of cell proliferation of NB cell lines by sulfasalazine, and computational docking of sulfasalazine into SPR. They provided well-conducted statistical analysis. The study is well designed, and the material and methods are appropriate. References are up to date and acceptable; the text is well written. I do not have major critical comments.

We thank the reviewer for the kind remarks. No response to comments is needed.

References: