Author's response to reviews

Title: The glutamate transport inhibitor DL-Threo-beta-Benzylxoyaspartic acid (DL-TBOA) differentially affects SN38- and oxaliplatin-induced death of drug-resistant colorectal cancer cells

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Version: 4 Date: 31 March 2015

Author's response to reviews: see over
Response to reviewer’s comments – MS 1601353759153108

Dear Ms. Remoto, dear Prof. Ciudad,

We have now revised this manuscript according to the insightful comments by the reviewers. Please find below our point-by-point response to the reviewers comments. A revised version of the manuscript has been uploaded.

We find that the revised manuscript is substantially improved, and hope that you will now find it suitable for publication in BMC Cancer.

Yours sincerely, on behalf of the authors,

Stine Falsig Pedersen
Professor

Point-by-point response to the reviewer’s comments. Our response to each point is indicated in italics.

Reviewer 1

Reviewer's report:
The authors have explored the role of the high affinity glutamate transporters SLC1A1 and SLC1A3 in the resistant phenotype of colorectal cancer cells made resistant to either SN38 or oxaliplatin. They addressed the question inhibiting these transporters with the nonselective inhibitor of EAATs, DL-TBOA and UCPH-101, a specific SLC1A3 inhibitor. Results obtained with UCPH-101 discarded SLC1A3 implication. However, authors could not directly associate the results to SLC1A1 because of the slight effect observed with the knockdown of this transporter and the lack of response observed when it is overexpressed. However, the title clearly shows the results obtained and the abstract explains what is displayed in figures, excluding the knockdown results. Moreover, discussion has been properly addressed, although authors should be careful with
statements that are not corroborating by statistical analysis. In page 14, line 299, authors declare that “p53 induction by chemotherapeutic treatment was consistently reduced”. In this sense, the statement of robust changes in the expression of SLC1A1 and SLC1A3 regarding the microarray analysis is excessive (page 7, line 114). Mainly, due to not all the results are validated by the following mRNA analysis.

Thank you for the appreciative words about our work. As suggested, we have now toned down the statements regarding p53 induction by chemotherapeutic treatment (p14) as well as the reference to the microarray analysis of SLC1A1 and –A3 (p 7).

Major compulsory revisions

Authors must refer the phosphorylation of pRb at Ser 807/811 to the pRb total protein levels instead of B-actin.

While we completely agree with the reviewer that it is generally best to normalize phosphorylated proteins to their total forms, this is frequently not done for precisely pRb, even in the most stringent high impact publications, presumably because of the high stability of this protein through the cell cycle (for examples, see Annicotte, J.S. et al Nature Cell Biology 11, 1017 – 1023, 2009; He, L. et al Nature 447, 1130-1134, 2007; Joseph, E.V. et al. Proc Natl Acad Sci USA 107(33):14903-8, 2010). Since our samples have now waited for over 4 months in the freezer because of the slowness of the BMC Cancer review process, we feel that it would potentially introduce errors to do such a normalization now, and since it is so often not done, we have taken the liberty of not doing it. We have, however, made careful corrections as per every single other point brought forward by the reviewer, and hope this will be acceptable.

When authors combine chemotherapy with DL-TBOA inhibitor, results show a slight decrease in p53 induction, although without statistics significance. However, when SLC1A1 is silenced, results don’t recapitulate the effect of DL-TBOA. Authors cannot assert that SLC1A1 knockdown partially recapitulate the reduction of p53 induction observe with DL-TBOA. Authors must obtain a higher knockdown of SLC1A1 transporter to demonstrate that the effect is specific of SLC1A1 rather than another transporter inhibited by DL-TBOA. Moreover, SLC1A1 overexpression has no detectable effect on p53, p21 or PARP induction what reinforces the idea that DL-TBOA effect doesn’t depend on SLC1A1.

We agree that given the lack of statistical significance and low knockdown efficiency, this point was overstated. Thank you for pointing this out. We have now rephrased the text on p. 2 l. 46 (abstract), p 11, l. 229-231) to make it 100% clear that the effect of DL-
TBOA may involve transporters additional to SLC1A1. This was already clearly stated in the Discussion (p 16, l. 332-333), which has therefore not been revised in this context.

Differences in SLC1A1 localization are difficult to established, because controls are in different figures. Figure 7 should be design again in order to clearly show the changes that are discussed. “Merge” should be changed to indicate f-actin staining.

Again, we agree, and have redesigned figure 7 accordingly. All the experimental conditions have now been collected in Fig 7, but only SLC1A1 staining is shown. A new Suppl Fig 7 contains the merged images with SLC1A1, F-actin and DAPI, which are not strictly necessary for the interpretation of the data but may be consulted for reference. We find that this rearrangement greatly facilitates the interpretation of the data.

Minor essential revisions
Page 2, line 45 and Page 21, line 466. Retinoblastomaprotein should be changed by Retinoblastoma protein.
Corrected

Page 10, line 205. Figure number of HCT116 parental cells results must be indicated.
Corrected (Fig. 5A and Suppl. Fig. 3)

Page 11, line 239. Authors should include the reference to Fig. 7B inside the Fig. 7A bracket and a reference to Fig. 7C when appropriated.
Corrected.

Page 20, line 448. Antibodyin should be corrected by antibody in.
Corrected

Figure 1. Molecular weight should be shown in western blot. Which SLC1A1 band has been quantified?
Corrected (and molecular weights have now been added to all blots, except for p53, p21 and p150, where it should be self-evident). The broad band around 65 kDa has been quantified. The broadness of the band reflects that the mature, glycosylated form of SLC1A1 is glycosylated.

Figure 1 legend. In section B, bottom and top should be erased.
Figure 6 legend. p21 and PARP1 are not showed in this figure.

Thank you – this is now corrected.

Reviewer 2

Reviewer's report

Major

Author show a down-regulation of SLCA1 at mRNA and protein level. They analize microarray data of CRC versus normal tissue and confirm that SLC1A1 is also down-regulated. A comparasion between CRC tumors from SN38 resistant and non-resistant patients could be an evidence of data obtained in cell lines. It sugest be done

This is a nice suggestion, and we agree that it would be very interesting. However, this would comprise a whole new study much beyond the scope of the present work. We have now mentioned this in the discussion (p 16, l. 347-48) as an interesting future perspective to the present work.