Reviewer’s report

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

Version: 3  Date: 17 March 2015

Reviewer: Sandra Pérez Torras

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.

Major compulsory revisions

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

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.

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.

Minor essential revisions
Page 2, line 45 and Page 21, line 466. Retinoblastomaprotein should be changed by Retinoblastoma protein.
Page 10, line 205. Figure number of HCT116 parental cells results must be indicated.
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.
Page 20, line 448. Antibodyin should be corrected by antibody in.
Figure 1. Molecular weight should be shown in western blot. Which SLC1A1 band has been quantified?
Figure 1 legend. In section B, bottom and top should be erased.
Figure 6 legend. p21 and PARP1 are not showed in this figure.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests