Reviewer's report

Title: Role of Caveolin 1, E-Cadherin, Enolase 2 and PKC alpha on resistance to methotrexate in human HT29 colon cancer cells

Version: 1 Date: 4 June 2008

Reviewer: Balazs Györffy

Reviewer's report:

Summary:
Selga et al identified the effects of the siRNA knockdown of selected genes on the MTX resistance in the HT29 cell line. The effect of the parallel knockdown of CAV1, ENO2, PKCa, and DHFR is more marked in the sensitive cells than in the resistant cells. The use of CAV1 siRNA and E-cadherin plasmide helped to reduce resistance and may represent therapeutic targets. The manuscript is well written and represents a value to the community.

Major Compulsory Revisions:

The microarray measurements and microarray data analysis is poorly described. Where are the microarrays in GEO (accession number?)? How many microarrays were all together made? Were there any replicates? Was there a false discovery rate computed (like SAM) or was there a multiple testing correction performed (like Qvalue)?

Since the used software (Genespring) is mainly for visualization of the data, a more robust statistical analysis (for example Rank Products in R) should be performed to detect differentially expressed transcripts. However, if less than 3 arrays per group were measured, then the use of a p value can be omitted and the use of fold changes might be a reasonable alternative - but in this case the microarray analysis is more „exploratory” than „significant” and this should be reflected in the interpretation of the results.

Minor Essential Revisions:

1. The results describing the effects of the siRNAs are also present on the Figures and thus the results section could be shortened to avoid overlapping.

2. There are too many figures:
   • Figure 6 is not informative and should be only mentioned in the text.
   • Table 2 is not informative and should be deleted.
   • Table 3 = Figure 7? Table 3 should be deleted.
   • Figures 3-4 should be combined in one Figure having A and B sub-figures, each having 4 columns parallel with different texture for the different siRNAs
• Figure 1 should be moved to the supplemental material.

Discretionary Revisions:

1. HT-29 is a colon cancer cell line. Currently, MTX is not used to treat colon cancer and this limits the implications of the study.

2. HT29 cells are usually hard to transfect. Was the transfection efficacy measured in any way?

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.