Reviewer's report

Title: RNA interference-mediated c-MYC inhibition prevents cell growth and decreases sensitivity to radio- and chemotherapy in childhood medulloblastoma cells

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Reviewer: Elena Chernolovskaya

Reviewer's report:

The manuscript under consideration describes the effects of c-myc targeted siRNA in a panel of medulloblastoma cell lines with different levels of c-myc expression. The authors show that silencing of c-myc expression inhibits cellular proliferation, clonogenic growth and G1-S phase cell cycle progression, and reduce hTERT expression and telomerase activity. The results further show, that down-regulation of c-MYC decrease the sensitivity of human MB cells to chem- and radiotherapy. The authors conclude, that “targeting c-MYC might be of therapeutic benefit when used sequentially with chemo- and radiotherapy rather than concomitantly”.

- Major Compulsory Revisions

1. Fig.1 A. It is not indicated which value is set as 1. In the figure legend is indicated “Values represent levels of c-MYC mRNA (n=3; ±SD) of control and c-MYC siRNA-transfected cells (y-axis; log scale) relative to control siRNA transfected DAOY wt cells” – but the value for control siRNA transfected DAOY wt cells is about 100.
2. Fig. 2 A. DAOY M2 – cell overgrowth, since that – poor inhibition.
3. Fig. 1B. – Quantification of the Western blot data is necessary, in the other case c-MYC activation data is useless, since the level of the protein can not be compared to its activity.
4. Fig. 2 D. The standard deviation should be indicated.
5. Figure legends contain sense repeats, needed to be reformulated.
6. Fig. 4 B. Markers should be presented.
7. Fig. 5 legend: It is not clear what “control cells” is used for the reference – non-transfected, non-irradiated or both? The MTS assay gives the number of living cells in the sample, but not the real viability (% of living cells as compared to the total amount of cells in the sample).
8. No indication about transfection and the type of transfection reagent in Materials and methods section.
9. Inadequate apoptosis assay is used or it is inadequately described. No internal control indicating equal amount of cells in the sample is included. Since c-myc gene silencing cause inhibition of cellular growth the number of the cells can vary
and sequentially the amount of the fragments can reflect the total amount of cells.

10. Most experiments (mRNA and protein level measurement, apoptosis and telomerase activity determination) was done at one defined time point and no explanation why this time point was taken is included in the article. The authors should refer to the literature data or to own observations.

11. Discussion, line 16 “c-MYC inhibition, independent of the c-MYC expression” – should be revised.

12. The difference in the levels of mRNA hTERT inhibition and telomerase activity inhibition is not discussed anywhere, may be wrong experimental time point or mutation in hTERT or any other reason?

13. Discussion, second page “c-MYC-mediated activation of hTERT gene expression and telomerase activity may contribute to c-MYC-dependent cellular immortalization in MB” – this statement is not supported by experimental data, see Fig.4: no activation in DAOY M2 compared with DAOY wt.

14. The difference in c-myc gene silencing on cisplatin and etoposide is not discussed, why in this case both drugs are presented?

- Minor Essential Revisions
The total percentage of D425 cells in different phases of cell cycle in Fig. 2C exceeded 100%, please check data.

- Discretionary Revisions
Medulloblastomas are represented by poorly differentiated neuronal cells and the goal of the therapy is to kill malignant cells by chemotherapy or IR or convert them to normal, and the agent of differentiation therapy (retinoids, resveratrol) are aimed at the second task. Since siRNA gives only temporal silencing and inhibition of tumor growth, it would be valuable to study the influence of c-myc down-regulation on the expression of markers of neuronal differentiation in the MB cells and the fate of the cells after c-myc reactivation. Massive apoptosis of osteosytes was detected by Jain M. and co-workers (Science 2002. V. 297 P. 102-104) after reactivation of c-myc expression.

Also, I would recommend to the authors to use other method for the detection of the apoptosis, which allows detecting live, dead and apoptotic cells in one sample

**Level of interest:** An article of limited interest

**Declaration of competing interests:**

I declare that I have no competing interests