Reviewer's report

Title: Neuronal markers are expressed in human gliomas and NSE knockdown sensitizes glioblastoma cells to radiotherapy and temozolomide

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Reviewer: Deepak Kamnasaran

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The article by Yan et al. investigates the functional roles of neuronal markers in human gliomas and further accompanies this research with survival, and radio- and chemo-therapy studies. Based on their precursory findings using a candidate gene approach, this group eventually narrowed their focus to studying the NSE gene. This body of work is important in our effort to understand the functional roles and with relevance to treatment responses of other categories of genes such as neuronal markers in astrocytomas, which is currently under represented in the field of molecular neuro-oncology. Overall, this manuscript can be improved with the following recommendations:

Major comments:

1. In the Results section, it is unclear what is the rationale to pursue further studies on the NSE gene based on the precursory findings. This needs to be clearly stated.

2. Findings from the real time PCR data cannot be clearly interpreted in Figure 2. It is difficult to follow what the authors describe in the results section and to correlate them with what is mentioned in this figure. The authors need to state what formula was used to calculate real time PCR fold changes in the Methods section. Furthermore, changes in the scales or values on the Y-axis can improve the presentation/interpretation of the real time PCR data.

3. Likewise, findings from the western-blot densitometric analysis (Fig. 2E) do not seem to correlate with what is visually observed in Fig. 2D and what statements the authors report in the manuscript. In the methods section, the authors need to report what formula was used to calculate the fold change in densitometric values. How many replicate experiments were done to arrive at this conclusion?

4. All knock down experiments are described as though they were undertaken with only one siRNA which targets the NSE gene. The datasets will benefit from more credibility if data is presented from a second siRNA that targets the NSE gene. Furthermore, in the methods section, the siRNA sequences and where in the NSE gene is specifically targeted needs to be stated.

5. Based on the data presented, it appears that a knocked down NSE expression is not significant to affect the migratory properties of glioma cell lines. This needs to be stated in the text instead.

6. A wide spectrum of NSE protein expression patterns were noted from the IHC
staining of human glioma biopsies. This section will benefit from a more descriptive reporting of what unique pathological hallmarks of the tumor specimens are correlating with unique NSE staining patterns like: 1) cellular location, 2) range of protein expression (% expression).

7. Although the datasets from the survival analysis is interesting, it is recommended that the findings from NSE vs GBM survival only to be reported. The number of specimens for low grade astrocytomas, and other types of gliomas is too low to provide accurate Kaplan-Meier analysis. Furthermore, it is unclear from the NSE vs CBM survival analysis what type of NSE staining patterns (nuclear vs cytoplasmic) were used in the Kaplan-Meier analysis. If the NSE staining patterns is stratified (nuclear vs cytoplasmic), what are the KM survival plots and do the datasets demonstrate significant differences?

Minor comments

8. In the legend of Table 1, it states that some patients were treated with CCNU; however, no samples listed in Table 1 were reported as being treated with CCNU.

9. Spell check to correct typographical errors in the text.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

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