Reviewers report

Title: Quantitative proteomic analysis shows differentially expressed HSPB1 in glioblastoma as a discriminating short from long survival factor and NOVA1 as a differentiation factor between low-grade astrocytoma and oligodendroglioma.

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Reviewer: Kai Stühler

Reviewers report:

The authors of the manuscript "Quantitative proteomic analysis shows differentially expressed HSPB1 in glioblastoma as a discriminating short from long survival factor and NOVA1 as a differentiation factor between low-grade astrocytoma and oligodendroglioma." performed proteome study on microdissected tumor tissue using 8-plex iTRAQ.

Major Compulsory Revisions

For the evaluation of the manuscript the authors are asked to provide a more detailed scheme of the experimental set-up. Which samples have been pooled in 8-plex iTRAQ and what was the applied protein amount etc.? Further the authors are asked to provide more demographic information about the analyzed patients as well as the genetic status about relevant prognostic markers of gliomas like e.g. IDH1 and MDGTM status. Otherwise the authors will not consider relevant subgroups of glioma which will have an impact on the subsequent data interpretation and stratification.

Another relevant issue is the selected criterion of 12 month and longer for long-term survival. From the side of the reviewer it is questionable if the selected criterion is wisely chosen if the median survival time for GBM is 10 to 14 months. It is therefore suggested to adapt the criteria to the common sense of the scientific community and considering the 3-5% of patients surviving more than three years (Krex et al., 2007) for long-term surviving.

Further critical aspect is the application of the suggested proteins (HSPB1 and NOVA1) as diagnostic marker. At the moment the analysis based on the statistical evaluation relying of high patient numbers showing that different groups exist. But for diagnosis it is relevant to answer the question how reliable can a patient assign to a specific group by measuring the marker protein. Therefore, for evaluation of diagnostic performance statistic like e.g. ROC analysis have to be applied allowing to present sensitivity and specificity of the chosen marker. From the shown data insufficient performance can be concluded relying on a high false-positive or false-negative rate dependently of the selected thresholds. The author have to consider this aspect appropriately.

Minor Essential Revisions

Figure 1 is unclear. Fold changes can only be calculated between and not within one group as presented in the figure (probably abundance?).
In Figure 3g detailed data for the GBM short and GBM long survival is missing.

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

**Quality of written English:** Acceptable

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