Reviewer's report

Title: Glioma stem cells are more aggressive in the recurrent tumor than in the primary tumor and they both can be maintained for long-terms in vitro

Version: 2 Date: 11 July 2008

Reviewer: Joerg Wischhusen

Reviewer's report:

This study addresses two issues that are highly relevant for glioma biology: On the one hand, the authors have succeeded in establishing new glioma cell lines that retain some features of glioma stem cells. And while others have done so, too (e.g. Beier et al., Cancer Res. 2007 May 1;67(9):4010-5), there is still a shortage of such cell lines, which makes this addition very welcome. What is quite special about this study is that the authors have generated their two cell lines from a primary glioblastoma and the corresponding recurrence 6 months later. Thus, a comparison of these two cell lines might allow an assessment of the therapy-induced or spontaneous changes that have occurred during this time period. Alternatively, the differences between the two cell lines might reflect the differences between glioma cells that either remain attached to the primary tumor or infiltrate the surrounding tissue where they survive the initial therapy and form the recurrent cancer. Thus, they may now be able to provide the scientific community with material and data of great interest.

Nevertheless, the manuscript is let down by a number of shortcomings. The following issues should be addressed in an appropriately revised manuscript:

1) The manuscript requires some language editing. Even though the authors manage to communicate their message quite clearly, there are too many unidiomatic phrasings, awkward sentence constructions, typing errors and other mistakes that should be corrected.

2) There is no indication of the treatment the patient received after surgery. This information has to be supplied – and it may also have implications on the interpretation of the data. Radiotherapy, for example, is known to result in a relative enrichment of stem cell-like glioma cells (Bao et al., Nature. 2006 Dec 7;444(7120):756-60.). Assuming that the patient has received standard treatment including post-surgical radiotherapy, the increased proportion of CD133+ cells in the recurrent tumor could thus be due to this treatment.

3) The characterisation of the glioma stem cells is rather incomplete. Thus, the data from the experiments that confirmed the self-renewal capacity should be presented. (What is the percentage of sphere-forming cells after dissemination into single cells? Does this percentage remain constant over various cycles?) Considering the differentiation potential: Is there a reason why the authors did not look for oligodendroglial differentiation, e.g. by staining against
galactocerebroside C?

Finally, the authors injected 200,000 cells into NC nude mice. Normally, much lower cell numbers should be sufficient if the cells were really tumor stem cells. In addition, such an experiment could give an idea, which proportion of the cells in these “GSC lines” really display tumor stem cell features. Thus, this information would be a required before the cell lines can really be considered GSC lines.

4) In Figure 3, it is not clear, what is shown in the inset. Is this the isotype control? In addition, it is very hard to depict a CD133-positive population in this FACS staining.

5) Obviously, the glioma stem cells only form a subpopulation of the cultured cells. Thus, it is doubtful whether the cell shown in Fig. 5 is really a GSC or not. It is not even clear whether this cell belongs to the CDD133+ subpopulation in the culture or was just picked at random. This should at least be discussed. In addition, the authors should be careful with the use of the term “glioma stem cells”. While it appears that they have established cell lines containing GSC, the actual GSC will only constitute a small fraction of the cell lines.

6) In Figure 6 C, error bars are lacking. Moreover, GAPDH is a poor reference gene when tumor cells are being compared with cultured normal human astrocytes. Due to the different metabolic activity, GAPDH expression may be hugely different between those two cell types. Thus, the data need to be confirmed using a further internal standard (like 18 S rRNA) or a combination of markers (Thellin et al., J Biotechnol 1999, 75:291-295.). This could also help to answer the question whether the very slight downregulation of Pten mRNA is statistically significant. In addition, some information on the controls and standards used should be added to the legend to Figure 6.

Minor points:

1) In Figure 2, the respective staining (e.g. anti-CD133) might be indicated in the figure.

2) In all figures, there is one label reading “Fig n” in black or white and another, smaller one indicating the unabbreviated “Figure n” in pink. One of these should be deleted.

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.

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