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: 15 May 2008

Reviewer: John Ohlfest

Reviewer’s report:

Minor essential revisions:

1) The title is still not grammatically correct. The words “for long-terms” should be changed to read “long-term”.

2) First sentence in intro, change “malignance” to “malignancy”. The paper is full of misspellings that should be corrected.

3) The paper is full of additional grammatical errors that should be corrected.

4) The authors should state what criteria they use in order to refer to a cell as a “glioma stem cell”. This should be clearly explained in the introduction of the paper. They attempt to define glioma stem cells in the first sentence of the discussion, but omit tumor initiation from this description, which is probably the most important criteria. Glioma stem cells should be defined based on their capacity to self renew, differentiate (which the cells in the current study failed to do), and tumor initiate. The expression of CD133 should not be listed as a requirement since several studies have identified CD133- glioma stem cells:

CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles.
Cancer Res. 2007 May 1;67(9):4010-5.

Identification of A2B5+CD133- tumor-initiating cells in adult human gliomas.

The authors do cite the cancer research paper in the discussion but state the data incorrectly. That is, autologous CD133+ and CD133- cells were not compared for tumor initiation in that paper.

5) Glioblastoma multiforme is misspelled as “multiform” in the first paragraph of the methods. This is just one of many spelling errors throughout the paper.

6) Fig3, the data should be shown as a histogram where the isotype control is overlayed on the CD133 stained sample.

Major Revisions:
1) The localization of CD133 staining shown in Fig 2 is not membranous (except for Fig2B), and therefore, probably just non-specific binding of the antibody because CD133 is a membrane bound protein. Moreover, the figure legend corresponding to figure 2 does not match the text where expression of GFAP and TuJ1 is referred to.

2) Figure 4 is not sufficient to show what the authors describe in the text. Figure 4b-d should be shown at 1-2X magnification so that the degree of infiltration in relation to the inoculation site is visible. As it is currently, the images are at too high of power to determine anything about invasion into the contralateral side.

3) The authors make a repeated effort to say that nobody has established a long-term glioma stem cell line. This is simply not true. Most importantly is the recent paper listed below where they established a series of glioma stem cell lines. Therefore, there is really nothing novel about the current paper.

Glioblastoma-derived stem cell-enriched cultures form distinct subgroups according to molecular and phenotypic criteria.

7) 4) The increase in the CD133+ fraction of glioma cells in recurrent relative to the primary tumor was first reported by Liu et al in 2006:

Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma.
Mol Cancer. 2006 Dec 2;5:67.

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

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

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