Reviewer's report

**Title:** Molecular analysis of non-culture CD133+ GBM cells revealed different signatures among high grade gliomas.

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**Reviewer:** Ana deCarvalho

**Reviewer's report:**

This manuscript describes dissociation, sorting, and molecular characterization of CD133+ and CD133- populations from eight freshly resected GBM tumors. The starting surgical material is appropriate, along with the clinical description. The parental tumors were characterized in relation to EGFR and PTEN copy numbers, and MGMT promoter methylation status. Results from gene expression profiling of sorted cells by Affymetrix, after RNA amplification, has been deposited in the appropriated database (GEO). From the Affymetrix results, 245 probe sets having present call for the 16 samples were used in unsupervised clustering, grouping the samples according to original tumor location. A list of genes differentially expressed between CD133+ and CD133- cells common for all eight tumors is presented. The authors than propose GBMs should be further sub-classified according to a group of 40 genes differentially upregulated in the CD133+ cells of a subgroup of tumors.

While characterizing GBM tumors based on molecular characteristics of never cultured CD133 positive cell population is an interesting approach, and groups of interesting differentially expressed genes in the data set have been identified, this manuscript will benefit from substantial revisions in the presentation and interpretation of the data.

**Major Compulsory Revisions**

1) From the Methods section, page 12: “RT-PCR was performed using specific primers to corroborate expression array result for several genes of those 245 presented probes (data not shown).” The inclusion of this data in the main body of the manuscript or as supplemental data will validate the Affymetrix array findings and enrich the manuscript.

2) Figure 1 is not clear. Particularly 1B could show FACS representation of percentage of CD133 positive cells.

3) Table 1:

3a).The meaning of the “Response” column must be stated in the text. What were the treatments each patient receive? Patients with “Complete response” presented a survival of 398 and 410 days.

3b)The percentage of CD133 positive cells would be more meaningful for comparison with other studies than the total number of CD133 positive cells in
each tumor.

4) From page 14, as it relates to genomic characterization of the tumors: “We have not found correlations with the biological features from patients”: this statement has to be more detailed and substantiated.

6) From page 11: “Statistical evaluation was carried out using the SPSS 15.0 statistical software. All P-values reported were two-sided and statistical significance was defined as P-values < 0.05”. Statistic analysis are not mentioned in the text or shown in the figures.

7) Other authors have published molecular profile studies comparing CD133+ and CD133- GBM cells. Citation of such publications and a more direct comparison with results presented here would be appropriate, even if some of these publications include CD133+ cells that have been cultured as neurospheres.

8) The approach used in this work, to obtain the molecular profile of sorted never cultured cells is a valid one. Manipulation of cells in culture leads to selection and alteration of gene expression. However, the authors should acknowledge in the discussion a couple of points. First, that the functional properties of the CD133+ cell population described here, such as the ability to self-renew in vitro, and to initiate orthotopic tumors in immunocompromised rodents has not been tested. Second that there are substantial evidence in the recent literature showing that the percentage of CD133 positive cells in GBMs vary widely, and that CD133 negative cells with properties of cancer stem cells have been described.

9) On page 18, “Additionally, differential expression of determined genes in CD133- vs. CD133+ population (mature glioma cell population), shown alterations in several genes involved in neural development disorders and previously described by other authors.” Supporting references should be included here.

10) On page 19, “endowed with metastatic characteristics”. Please review this statement, as glioblastomas do not typically metastasize.

11) In the last section of the Results, entitled: “Two different GBM groups can be functional defined according to the expression pattern of 40 genes”, Figure 6 clearly show de differential expression for the two CD 133 positive GBM samples (G4 and G7), and the authors state that “We hypothesize that these these up-regulated gene levels could differentiate 2 diverse kind of GBM, one less proliferative and the other one endowed with metastatic characteristics (G4 and G7) respect to the others”. Since this is just a hypothesis that needs further proof, one is lead to question if the title of the paper convey the main findings.

Minor Essential Revisions

1) This manuscript will benefit of through revision of typos and language. Here
are some examples where the problems are underlined:

Abstract:

“Besides, we have completed the genomic study of these tumours by compared genomic hybridization (CGH) arrays, FIS studies”

“Primary glioblastomas could be sub-classified accordingly to the properties of their CD133+ cells”

“molecular characterization of these stem populations could be critical to found new therapeutic targets”

Page 8, “flow citometry”, “Possible mature red cells were depleted”

Page 10: “All DNAs were quantities using the Nanodrop”

Page 12: “Specimens were considered amplified for EFGR when more”

Page 13: It was interested that only those two patients with higher number of CD133+ cells (higher than 1%, more than 10,000 cells) were not able to response to treatment”

Page 16 “whereas the rest presented temporal or local locations”

Page 16: “Gene expression profile of both CD33+ and CD133- cells using microarrays

was arranged to establish possible answers between clinical correlations and gene expression.”

Some references need reformatting: e.g. Refs. 28, 29, 32,

2) Abstract: should clearly state the goals of the work in the background section. The methods section should include the gene expression profiling and analysis employed.

3) Figure 2: significance of the data presented in this figure would benefit from more details.

4) Figure 3 legend: “we compared the gene expression profiles of purified CD133+ cells from GBM patients versus CD133-cells from control mice.” Is this a typo?

5) From page 8: “Cell suspension was gone through 0.7 μm single-cell filter”. Please review, as this pore is too small for a mammalian cell.

6) Was there any enzymatic step in processing the tumors to obtain single cell suspension?

7) From page 13: “some groups have publish the relationship between the high CD133+ cell number and their resistance to therapy in patients [28, 29]”: Please check these references, as ref 29 does not seem to related clinical data.
8) From page 16, “Gene expression profile of both CD33+ and CD133- cells using microarrays was arranged to establish possible answers between clinical correlations and gene expression. However, no relevant connections were found between these 2 parameters (gene expression and biological-clinical correlations).”. This statement needs to be more substantiated by detailed explanation of data analysis performed to establish correlation between clinical outcome and gene expression.

9) Supplementary data -CGH-methods: “Additionally we applied the binary segmentation method described by Olshen et al.”: please include full reference.

10) Supplementary table: additional labeling/explanation of values listed on columns would be appropriate.

Discretionary Revisions:

1) From the introduction: “The cancer relapse and mortality rate suggests that current therapies do not eradicate all malignant cells. In this sense, there is increasing evidence that many types of cancer contain their own stem cells: cancer stem cells (CSCs), which are characterized by their self-renewing capacity and differentiation ability [1].”

Specifying the types of cancer referred to and including additional relevant reference would be appropriate here.

2) Titles for Tables 2 and 3 (e.g. “Common up-regulated in CD133+ vs.CD133-GBM cell patients.”) could be more descriptive, e.g. including the word “genes” and excluding “patients”

Level of interest: An article whose findings are important to those with closely related research interests

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.