Author's response to reviews

Title: Characterization of cells recovered from the xenotransplanted NG97 human-derived glioma cell line subcultured in a long-term in vitro

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Author's response to reviews: see over
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August 20, 2008.

Dear Ms Editor Dr. Norton,

Enclosed is a manuscript titled “Characterization of cells recovered from the xenotransplanted NG97 human derived glioma cell line subcultured in a long-term in vitro” with alterations suggested by referees.

We have numbered all referees’ suggestions as presented, followed with the explanations about the alterations as described below:

Reviewer: Randy Jensen
Discretionary revision suggested from referee:
1) The introduction should describe that most patients with these tumors are dead by two years.
2) The introduction is too long and unfocused, some of it could be moved to the discussion section.
3) I am not sure what value figure 1 has when the essential information is contained in Table 1.
4) I can't tell what figure 2b is for, this should be described better in both the figure legend and the results section.
5) The discussion should be broken up into smaller subsections for reader use.
6) The discussion should contain a paragraph or two discussing the limitations of having this tumor grown originally in the mouse flank. It should also be discussed that vimentin and GFAP expression in GBM is highly variable from
patient to patient.

Minor Essential Revisions suggested
1) The calculation of the doubling time does not make any sense, this needs to be clarified.
2) What is being shown in figure 3? I don't see a change in either GFAP or vimentin expression over time. This is even less convincing with the 40kDa band in one lane and 57 kDa bands in the other, what is going on here?
3) Figure 4 should contain panels all at the same magnification. If they are trying to demonstrate decreasing expression of GFAP and increasing Vimentin over time, having the cells at different magnification does not allow for the reader to make this distinction.
4) Please make a distinction in the results section of what is different between figures 6 and 7
5) Discussion of why the immunohistochemistry was only done on passages 19-83 should be made, why not include passages 5-19 or 84-100?

Our comments:
Discretionary revision

1) We rewrote the background and we added about the follow up of two years for the GBM patients.
2) We rewrote the introduction (background) in order to correspond to referees suggestions, but we believed that this topic must contain important concepts that situates the reader and will allow proper conclusions.
3) We agree that some essential information from figure 1, doubling time decrease, was presented in table I, however all graphics ensure that the cells cultivation did not suffer any external stress that would change their cellular characteristics and modulate these cells to a malignant phenotype. Moreover, these stable culture growth graphics guarantee that possible cytogenetical alterations are not due to media alterations.

4) We added to the 2b legend and results necessary information to elucidate the figure representation.

5) We broke the discussion as indicated by referee.

6) We added a paragraph that discusses more about GFAP and Vimentin in GBM in cells cultures and patients.

Minor Essential Revisions
1) We rewrote the results to the doubling time calculation to make more sense to the reader.
2) The figure 3 was rediagrammed to clarify our results.
3) The figure 4 was also rediagrammed.
4) We made a distinction in the results on figures 6 and 7.
5) We added at discussion a sentence who describes why we used those cell passages. We also distinct the flow cytometry for three passages and present individual percentage of each marker and the average of cytometry flow positive cells. About the cytogenetics we did alterations in material and methods to explain our measures and the cytogenetical analysis in NG97 cells were not available any more because we have technical problems that compromised the cytogenetical results with NG97 cells.

Reviewer: Ivana Magnani

Major Compulsory Revisions suggested:
1) Consider to delete from “A few study(line 4)……… to malignant phenotype” (line 8).
2) The authors didn’t investigate the “role” of ontogenetical characteristics(?), adhesion molecules and chromosomal instability. They studied the localization by immunocytochemical and Western blot analysis of specific antibodies. Thus the discussion is too much speculative about the role of markers such as GFAP,
Vimentin and Integrins during in vitro progression of NG97ht cell line.

3) Chromosomal abnormalities detected in NG97ht are not specified. How do the authors conclude that cytogenetic analysis shows structural and numerical abnormalities of grade III/IV astrocytoma?

4) Given that NG97ht karyotype retained human rearranged chromosomes, they could ask if such chromosome markers were already present in the original culture before the xenotransplant. The acquisition of these data might improve the understanding of the karyotype evolution in vivo. To disclose cryptic derivative chromosomes FISH analysis could be employed.

5) The authors should adequate the conclusion by the data.

Minor Essential Revisions suggested:

1) Include the Country’s name in the affiliations

2) Write correctly “NG97(ht)” in the text Abstract:

3) consider to start with “Our laboratory set up…”

4) consider to cancel last sentence “This work….”

5) Keywords: consider to delete “glioma” and “tumor progression” and include in “vitro progression” and “immunodetection”.

6) Introduction:

- The beginning is too long: the authors should start with the explanation that established cell lines are useful tools to study the tumor progression, giving literature data on glioma.
- The sentence “Cells from the tumor mass (NG97ht) were then processed and cultivated in vitro as an adherent monolayer and had the same morphological characteristics of the original culture before the xenotransplant “ doesn’t constitute the background. It sounds the aim of the work and could be moved at the end of the background.
- Add references about “in vivo cell fusion in cancer”.
- Consider to cancel the sentence “In the endeavor… till “this cell”.
- As regards the last sentence: the results here reported didn’t “explain” the “tumor spontaneous progression of the astrocytoma”

7) M&M:
- Consider to delete: expression of GFAP and vimentin/ evaluation of integrins/ evaluation of integrins ligands
- Replace “Immunohistochemistry” with Immunocytochemistry

8) Results:
- complete Fig. 3 with a molecular weight marker
- include in: Results of GFAP and Vimentin expression the alphabet letters of Fig.4

Our comments:
Major Compulsory Revisions suggested:
1,2,3) We rewrote the text and added some references to fill these suggestions.
4) We rewrote this part of discussion.
5) We rewrote the conclusion.

Minor Essential Revisions

1,2,3,4 and 5) We rewrote the text to fill these suggestions.
6) About the introduction (background) we rewrote and added some references in order to improve the manuscript file.
7,8) We altered these topics to adequate the text.

We considered positively all the suggestions mentioned to clarify our manuscript. We appreciate all suggestions from referees. Please contact us if you have any further questions. We look forward to hearing news from our manuscript soon.

Best regards,
Camila Machado.