Reviewer's report

Title: Notch signaling contributes to the maintenance of both normal neural stem cells and patient-derived glioma stem cells

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Reviewer: Christi Kolarcik

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The authors utilize a cell culture and molecular biology-based approach to characterize the effects of inhibitors of the Notch signaling pathway on patient-derived glioma stem cells (GSCs). Comparisons are made to mouse embryonic brain-derived neural stem cells (NSCs). Although the role of cancer stem cells and the potential therapeutic significance of Notch inhibitors are of great interest, there are some concerns with the current manuscript.

Discretionary Revisions:

1.) The authors could detail the primers utilized in table format.

2.) Additional references could be included in the Introduction. For example, in the first paragraph, the following sentence could be supported by specific research articles: “Notch signaling, an evolutionarily conserved pathway mediating direct cell-cell interaction, has been shown to regulate neural stem cells (NSCs) and glioma stem cells (GSCs) in normal neurogenesis and pathological carcinogenesis, respectively.”

3.) The scale for the y axes in Figures 7A and 7B should be the same.

Minor Essential Revisions:

1.) It is unclear what the * and ** mean in the figures. Although it is sometimes stated in the text, this information should be included in all of the captions.

2.) The explanation of the markers used to differentiate stem cells from INPs is first discussed in the “blockade of Notch signaling promotes the conversion of GSCs to INP-like cells” when this should be addressed earlier in the “blockade of Notch signaling attenuates the proliferation and self-renewal ability and promotes differentiation of normal NSCs” section of the Results.

3.) The method for obtaining the mouse embryonic brain-derived neural stem cell cultures is not described in the Methods section.

4.) The authors indicate in the “blockade of Notch signaling attenuates the proliferation and self-renewal ability and promotes differentiation of normal NSCs” section of the Results that 100 cells per sphere are observed with GSI treatment. The number of cells per sphere observed with DMSO treatment should also be reported.
5.) Some of the results are overstated. For example, in the third paragraph under the “blockade of Notch signaling attenuates the proliferation and self-renewal ability and promotes differentiation of normal NSCs” section of the Results the authors conclude that inhibition of Notch signaling in NSCs leads to accelerated differentiation. A more accurate description would be that inhibition of Notch signaling leads to an increase in the number of differentiated cells.

6.) The n for each of the reported percentages of differentiated versus non-differentiated cells must be reported.

7.) Confocal microscopy should be used to confirm the existence of the “double positive cells”.

8.) The authors should elaborate on the clinical implications of the observed early GSI-resistance.

Major Compulsory Revisions:

1.) In the “glioma samples” section of the Methods the authors state that “independent cultures from at least three samples were used for each experiment”. Does this mean that all 9 patient samples were not used for each portion of the study? Were the 7 patient samples from which proliferating neurospheres could be obtained utilized? If not and if different specimens are used for different experiments, rationale for this decision should be included (i.e., each grade was represented, similar results were obtained irrespective of the sample, etc.) and the patient specimens used for each experiment specified.

2.) Related to the previous concern, the link between tumor grade and the frequency of tumor stem cells is addressed in the Discussion. The authors have not provided data to support this claim. In fact, the studies using patient-derived GSCs fail to identify which patient samples are being represented and the number of counts/patient. It would be valuable to know which of the patient samples (and their corresponding grade) were utilized. In addition, there is no attempt to explain why only 7 of the 9 specimens gave rise to proliferating neurospheres or even which samples did not give rise to neurospheres. If this information is provided, perhaps the relationship between tumor grade and stem cell abundance could be properly considered and evaluated.

3.) The methods used to count and measure the neurites needs to be described further in the Methods. Are the numbers and lengths of neurites reported in Figures 5B and 5C averages or totals per neurosphere? The authors indicate that 30 neurospheres were counted; however in Figure 5B there are only 12 symbols per treatment shown on the graph. In addition, the authors state that “more and longer neurites” were, in part, “demonstrated by estimating” which is problematic.

**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.