Reviewer's report

Title: Regulation of sonic hedgehog-GLI1 downstream target genes PTCH1, Cyclin D2, Plakoglobin, PAX6 and NKX2.2 and their epigenetic status in major brain tumors

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Reviewer: David Walterhouse

Reviewer's report:

The bottom-line message of the manuscript still needs clarity. The authors have improved the background section (although I believe it can still be shortened), which now points out their reasoning for studying the five genes in astrocytoma and medulloblastoma. The only experiment, suggesting a causal relationship between GLI1 expression and alteration in target gene expression is the siRNA experiment. The remainder of the gene expression data are descriptive and show varying results between samples. This variability between samples is not adequately discussed and remains troubling to me. Based on the varying results it becomes difficult to know the significance of the observations as presented. The links between the epigenetic studies and the gene expression study still need to be clarified to make the paper cohesive.

Major Compulsory Revisions

1. Although the English has been improved throughout the manuscript, the entire manuscript still needs editing and shortening to improve understanding.

2. A very specific conclusion should be included. Based on the current presentation, and variable nature of the results, I am not sure what the specific conclusion is. The only genes that show the same pattern of regulation in the siRNA experiments in medulloblastomas and astrocytomas are GLI1 and Ptch1, and regulation of Ptch1 by GLI1 is already well known. If GLI1 plays roles in regulating any of the other genes that are studied, then GLI1 appears to play different roles in different settings (even in the same type of tumor). An expanded explanation for the variable results should be provided, e.g. including the possibility that HH signaling may play a role in the development of some medulloblastomas and maybe some astrocytomas and not others; the possibility that GLI1 is not regulating the "targets" that are studied and that the patterns are associations. Indeed, pitfalls are not really discussed.

4. I really don't see a firm conclusion based on the epigenetic studies; this should be added.

5. The methods state that qPCR experiments were done in triplicate. I don't always see error bars in the figures. Why aren't the results of normal human brain compared statistically with the experimental samples?

6. p. 24 "PTCH1 expression in medulloblastoma: It might be more likely that low
PTCH1 in the setting of high GLI1 indicates that HH signaling is not active in that setting rather than the PTCH1 has inhibited the pathway.

7. Throughout the discussion the authors suggest causal relationships between GLI1 expression and expression of potential targets. I do not feel that the data are strong enough to support these statements, in view of the variable results.

Minor Essential Revisions

1. I would remove "major brain tumors" from the title and replace this with "medulloblastoma and astrocytoma."

2. I would still STRONGLY recommend shortening the manuscript considerably (including the introduction, results, and discussion) to bring clarity to the message. The overall message gets lost in the presentation; especially in view of the variable results.

3. There are still spelling errors - e.g. astrocitoma.

4. In the results section, no interpretation is provided for figure 1A-H. I do not think that figure 1G and H are necessary.

5. In the results section,"GLI1 may up-regulate PTCH1 in medulloblastoma and astrocytoma", some of the data appear to have been previously published (reference 26). The authors should clarify whether the same data are used in the figures in this manuscript as in reference 26 or whether the same experiments were conducted again.

6. p. 20 - From the data presented, it is not possible to conclude that GLI1 upregulates NKX2.2 which in turn inhibits PAX6. The data are descriptive and not causal.

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

**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.