Reviewer's report

Title: Regulation of sonic hedgehog-GLI1 signaling mediated downstream target genes PTCH1, Cyclin D2, Plakoglobin, PAX6 and NKX2.2 and their epigenetic status in astrocytic cell lines and primary tumor samples

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Reviewer: Rebecca Ihrie

Reviewer's report:

The revised paper still lacks a central testable hypothesis, and seems to be largely descriptive. More problematically, the authors assert causation where minimal evidence (or correlation) seems to exist. The expression analysis shown seems to indicate that expression of Hedgehog target genes varies widely between tumor cell lines and between individual tumors, and high levels of expression of one gene do not correlate to the levels of expression of another. It’s also unclear how the protein expression data and promoter methylation analysis in the later figures correlate with patterns of target gene expression. Are the authors suggesting that, in cases where Gli1 is highly expressed but Ptch1 or Cyclin D2 are not, that promoter methylation is the primary regulatory mechanism? If so, the samples where this occurs should be much more clearly indicated in the figures and text.

Additionally, the authors suggest that the low expression of Ptch1 in some Gli1-expressing cell lines and tumor samples is due to its role as a negative feedback regulator of the Hedgehog signaling pathway. It’s unclear if they are trying to argue that Ptch1 transcription is blocked through some alternative mechanism, allowing continuous pathway activation, or that low Ptch1 transcription means that the pathway, although activated to high levels, is also eventually repressed. In either case, no clear model is presented to explain the observed disparities between Gli1 transcript levels and Ptch1 transcript levels.

Major Compulsory Revisions:

1) The major caveat to the conclusions of the paper is that no statistical validation is given for the correlations that the authors assert. Without a meaningful statistical analysis, it is not possible to determine whether levels of Gli1 expression (or promoter methylation) actually correlate with levels of expression of other target genes. The conclusions the authors present would be best supported by unsupervised clustering or similar analysis, as suggested in the previous round of review. Although the authors state that they used a t test to determine significance, this test is generally used to compare between two populations and is unsuitable for the kind of analysis of multiple variables that they seek to complete.

2) The language of the paper still contains significant grammatical errors which make it difficult to understand.
Minor Essential Revisions:

1) In Figure 1, images from both the GFP channel and phase-contrast should be shown for all samples, to allow readers to see the approximate percentage of transfected cells and to be sure that untransfected cells are not autofluorescent.

2) Figure legends for Figures 2 and 6 should be corrected and clarified. For example, the description of 2E and 2F describes a “113% decreased” in gene expression when expression actually increased. The latter half of Figure 6 is also extremely unclear – graph labels are not legible, and it is not always clear which samples are being tested.

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

**Quality of written English:** Not suitable for publication unless extensively edited

**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.