Reviewer's report

Title: Curcumin-induced HDAC inhibition and attenuation of medulloblastoma growth in vitro and in vivo

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Reviewer: Anat Erdreich-Epstein

Reviewer's report:

Authors provide data that describe the effect of curcumin on medulloblastoma in terms of effect on apoptosis and HDACs, and in two in vivo mouse models. A large body of data already exists regarding the effect of curcumin in cancer models in vitro and in vivo, including two prior papers that show that curcumin induces apoptosis in medulloblastoma cells and a number of papers showing its effects on HDACs. Thus, the main novelty of this paper is in showing the effect of curcumin in two in vivo medulloblastoma models.

Major compulsory revisions:

Results: Second paragraph, authors describe that curcumin-induced apoptosis was inhibited by z-VAD-fmk, but do not show the data: this needs to be shown.

Results: A number of western blots are lacking a panel for a housekeeping gene to ensure equal sample loading (e.g., Fig 1C, Fig 5B, Suppl Fig 3). Additionally, when examining phosphorylation of proteins it is necessary to show the total amount of the protein on the same blot (e.g., Fig 5C, HDAC4/5/7 phosphorylation requires panel of the same blot probed for these HDACs).

Results: In several figures authors have shown measures of apoptosis (PARP and caspase 3 cleavage etc). However, in Figure 2A the panels do not show any accumulation of cells in the sub G1/G0 phase, even at 24 hrs. How do the authors reconcile these data?

Results: bar graphs should represent means of at least three independent experiments, and should include error bars to demonstrate the variability between experiments. Similarly, experiments showing an effect in a microscopic field need to have an accompanying panel that quantifies the effect in a number of fields over at least three separate experiments and provides the means and error bars. Preferentially, these quantifications should be done by at least two independent blinded observers.

Results: Demonstration of an anti-tumor effect of curcumin two in vivo models is the major strength of this manuscript. However, the p-values in Fig 6A (subcutaneous tumor model) are all >0.05. The authors used t-tests to compare tumor measurements at three time points to tumor size in the corresponding control time points, which may not be the optimal method for assessing these differences. Consultation with a statistician will likely permit use of a more
efficient (hence more powerful) statistical analysis for this experiment, and thus better evaluate the differences in tumor growth.

Major discretionary revisions:
Discussion: Authors state in the first paragraph that they have demonstrated that curcumin induces apoptosis in medulloblastoma cells “BY” reducing HDAC4 etc. However, although they showed that both apoptosis and reduced HDAC4 do occur following curcumin treatment, they have not demonstrated that the HDAC4 decrease mediates the apoptosis. In the third paragraph of Discussion they further elaborate on this potential causal relationship but do not show data to support it. Addition of mechanistic experiments to examine the causal relationship between curcumin, HDAC4 and apoptosis will significantly enhance this manuscript. Alternatively, they should amend the language in the relevant sections.

Minor essential revisions:
Background: Last sentence in second paragraph of Background is not clear.

Background: Please add references to support that curcumin crosses the BBB (first sentence in third paragraph in Background). Also, last sentence in that paragraph implies that the BBB is “one of the major obstacles for chemotherapy in pediatric brain tumors”, ignoring the fact that although some drugs can not cross the BBB, many chemotherapy agents sufficiently enter the CNS and are in fact used in therapy of medulloblastoma (e.g., CCNU, cisplatin, etoposide, vincristine, cyclophosphamide, thiotepa, carboplatin). Please adjust the language.

Background: In the first paragraph of the Background section, the authors should choose references that directly address the statements they provide when introducing medulloblastoma incidence, prognosis and treatment outcome. In the present version the two first references address the points they seek to support only indirectly or in quoting other references.

In Methods, when describing preparation of lysates for the various methods (in vivo, HDAC, immunoblotting), authors indicate both 1% and 0.1% Triton X-100: please verify that this variation is not due to a typographical error.

Results: in the first paragraph authors state that the morphological changes (shrinking, rounding, detachment) ‘indicate’ that curcumin induces cell death. Although such morphological changes can accompany cell death, they do not definitively indicate its presence.

Results: third paragraph, first/second line: there is no need to explain to readers in the cancer field that carcinoma is a cancer derived from epithelial cells.

Discussion: Parts of the discussion are repetitious and should be significantly trimmed, especially in sections that have only background, with little direct connection to the work presented. Additionally, all but the last two sentences in “Conclusions” consist of only background that has already been presented and
should be omitted.

**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:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests