Reviewer's report

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

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Reviewer: Caroline Saucier

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In this study, Lee and colleagues have addressed the anti-cancer potential of curcumin in medulloblastoma. Their data nicely convey that curcumin induces apoptosis and cell cycle arrest at the G2/M phase in medulloblastoma cells. These effects of curcumin treatment correlated with a reduced HDAC activity and HDAC 4 protein level, as well as an increased in tubulin acetylation and mitotic catastrophe. Of particular significance, they showed that oral delivery of curcumin in mice inhibits medulloblastoma xenografts tumor growth and prolonged the survival in Smo/Smo transgenic medulloblastoma mouse model. The manuscript is overall well written, scientifically sound and comprehensive to the reader. The data presented convincingly support the value of curcumin as a potential therapeutic agent for medulloblastoma. However, some issues need to be addressed by the authors.

MAJOR COMPULSORY REVISIONS

1) I recognize the effort of the authors to provide mechanistic basis for the anti-tumor effect of curcumin in medulloblastoma. I concur with the authors that curcumin treatment reduces HDAC activity. However, to conclude that curcumin-mediated reduction in HDAC activity and anti-tumorigenic effects is mainly caused by decreasing specifically HDCA4 expression is premature and a bit misleading. In fact, HDAC2 and HDAC7 appears to be reduced, particularly at earlier time points (Fig. 5B). Likewise, HDAC2 protein level is also reduced in D283 and D341 medulloblastoma cell lines treated for 24 hrs with curcumin (Supplemental Fig. 2). While the levels of HDAC activity and HDAC4 protein were measured as early as 3hr after curcumin treatment, the protein levels of the others HDAC isoforms were assessed at much later time point (15 and 24 hr). Measuring the expression levels of the other HDAC isoforms at earlier time point (at 3 hrs) might be of value. Likewise, it is a bit misleading to conclude that curcumin treatment reduced phosphorylation of HDAC4 in medulloblastoma cell lines based on the data shown in Fig. 5, where multiple bands were detected. The phospho-specific HDAC antibody used recognized not only phosphorylated HDAC4, but as well as the HDAC5 and 7 isoforms, which all have relatively similar MW (120-140 kDa). Hence, I suggest the authors to tone down the claim that curcumin effects in medulloblastoma cells are mediated by reduced HDAC4 expression and activity within the abstract and the discussion.

2) The authors propose that HDAC inhibition in curcumin-treated cells contributes
to the induction of apoptosis rather than being a byproduct of apoptosis. The authors should attempt to validate this hypothesis by measuring the HDAC activity and HDAC4 (and others HDAC isoforms) protein levels in cells treated with z-VAD-fmk, an inhibitor of caspases, which seemingly is blocking curcumin-induced apoptosis (data not shown). Demonstrating that HDAC activity and HDAC4 protein levels are still reduced in presence of caspases inhibition would provide support for this hypothesis. Conversely, it might provide mechanistic basis by which curcumin mediates reduced HDAC4 expression, since caspases were shown to mediate specific cleavage of human HDAC4 (Liu et al. J. Biol. Chem. 2004, 279:34537-46 and Paroni et al., Mol. Biol. Cell 2004, 15:2804-18).

3) Fig 1C, please provide, protein loading control.
4) Fig 2B, for the quantitative analysis of cell cycle profiles, please provide statistics.
5) To facilitate interpretation, please indicate the MW standards for each immunoblot analysis and the expected MW of HDAC protein isoforms analyzed.
6) The authors should discuss the possible mechanisms (e.g.: mRNA and/protein stability) by which curcumin mediates loss in HDAC4 protein (and of other HDAC protein isoforms).
7) In supplemental Fig. 3, curcumin seams to induce the appearance of an HDAC phosphorylated species in the nucleus. The nature of this HDAC phosphorylated protein should be discussed.

MINOR ESSENTIAL REVISIONS
1) Please, provide a brief description of the Smo/Smo mice models.
2) Fig 1D, please indicate in the legend the duration of curcumin treatment.
3) Fig 4A, Fig 5A & D, please indicate in the text and legend the time of curcumin treatment.
4) In Fig. 6A and its legend, please indicate when curcumin treatment was initiated.
5) Page 5, line 21, "results. For" instead of "results, For"
6) Page 7, 2 paragraph, line 8, space before Alexa
7) Page 13, last paragraph, line 4. Add comma after cells
8) Page 21, line 11, add a point after [10].
9) Page 22, "Curcumin has been used….. clinical trials in adults." and "No adverse …..reported so far." Please add references.

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

Quality of written English: Acceptable
**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