Reviewer’s report

Title: Curcumin analogue T83 exhibits potent antitumor activity and induces radiosensitivity through inactivation of Jab1 in nasopharyngeal carcinoma

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Reviewer: Peter Daniel

Reviewer’s report:

The authors study the anti-tumor effect of a curcumin analogue, T83, in a nasopharynx carcinoma cell line, CNE2. They show that T83 induces cell cycle arrest in a tetraploid state and induces apoptosis with processing of caspase-3. Moreover, they show that the radio-resistant subline CNE2R shows a high sensitivity to T83 and a more pronounced down-regulation of the c-Jun activation domain-binding protein-1 (Jab-1). To establish a functional role of Jab-1 in regulating sensitivity to T83 they perform siRNA experiments and show that Jab-1 siRNA reduces clonogeneic survival and increases apoptosis.

As it stands, the data are performed only in a single cell line system with a limited set of analyses. More-in depth signaling analyses and mechanistic insights would be desirable. The following points need to be addressed:

Major points:

1. The authors should state whether the CNE2 cells are of human origin and how they confirmed the cell line identity.

2. Evidence should be provided that the CNE2R cells are in fact a subline of CNE2 and not an unrelated cell line contaminant.

3. The authors should show the therapy effect of T83 in vitro in relation to the activity of curcumin.

4. At least one additional cell line should be studied with regard to T83 activity and the role of Jab-1 to show the broader relevance of these findings.

5. A second, independent apoptosis assay should be performed, e.g. Annexin-V-FITC/PI double staining.

6. The functional role of Jab-1 should be put on a broader experimental basis. The authors should show that T83/Curcumin/Jab-1 siRNA affect aspects of Jab-1 signaling such as, e.g. regulation of p27KIP1 and stabilization of complexes of c-Jun or JunD with AP-1 sites.

7. The authors show that CNE2 cells arrest in a tetraploid "G2/M" stage. Do they have information on the p53 status of CNE2? Failure to activate p53 and p21CDKN1 would facilitate arrest in G2/M.

6. In the same vein: JAB1 is also known as COP9 signalosome subunit 5 (CSN5), which is a component of the COP9 signalosome regulatory complex (CSN). The COP9 signalosome has been shown to regulate p53 stability. Would
T83/Curcumin/Jab-1 siRNA affect expression of p53 protein and induction of p21CDKN1? Such analyses would provide more mechanistic insights.

Minor points:
1. typo y-axis label figure 2B: apo(p)totic
2. The shading of the bars in the figures is a bit unfortunate. It is recommended to use black versus white bars as the current shading is not easy to differentiate in all figures.

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.