Reviewer's report

Title: Embelin inhibits TNF alpha converting enzyme and cancer cell metastasis: Molecular dynamics and experimental evidence

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Reviewer: Prem Sinha

Reviewer's report:

Embelin has been well demonstrated to possess anti-tumor activity on a variety of cancer cell lines. Such anti tumor activities either comes from inhibition of cell growth promoting signaling pathways or from induction of apoptosis by affecting X-linked inhibitor of apoptosis, an anti-apoptotic protein. In the present article, the authors shows that embelin docks into the active site of TNF-alpha converting enzyme (TACE). Further, the author shows that embelin treatment results in reduced protein expression of TACE in breast cancer cell lines along with inhibition of cancer cell growth and metastasis.

The manuscript shows that embelin inhibits cancer cell growth and metastasis through inactivation of metastatic signaling molecules. However, the evidence to link embelin and TACE is mainly based on Molecular docking at present and further experimental evidence is needed to substantiate the proposal that biologically embelin act as a ligand for TACE. Below are some suggestions that will be helpful in improving the quality of the work:

Major Compulsory Revisions-

1. In docking experiments, the authors demonstrate that embelin has an affinity to bind to TACE. Thus, one should expect to see the change in activity of TACE in converting pro-TNF-alpha to TNF-alpha. The authors should carry out TACE activity assay to demonstrate that indeed it is the TACE activity that is being affected by treatment of breast cancer cells with embelin.

The anti-proliferative effect, reduced colony formation in embelin treated cells might result from its effect on various signaling pathways or from its anti apoptotic effect. To provide the evidence that the effects of embelin in treated breast cancer cells are through TACE in Figure 3A, 4 and 5, one has to perform rescue experiments by overexpressing TACE.

2. It would be worthwhile to see the active TNF-alpha expression in these breast cancer cells either in unstimulated condition or under condition when cells are stimulated with some activators, which are known to induce TNF-alpha expression in the presence or absence of embelin.

3. There seems to be a quite a few errors either in the text or in the figure labels, while describing various residues of TACE. The authors should carefully check and correct all the errors in writing/numbering various residues of TACE both in
the text and in figure labels. [For example, in the section of Methods describing “Molecular docking” (page7, line 151), Leu348 and Gly349 are written as Gly348 and Val349. His406 is written as Glu406 in the part describing “Identification of the…..TACE protein” in Results section (page12, line 274). Similarly, in Figure1C, Glu406 and His415 are labeled as His406 and His15]. Further, it will be easier for one to follow the figures if the color-coding of critical residues remains same in Figure1C and Figure2B.

4. The “Discussion” section primarily centers on Molecular Docking results. Inhibition of cancer cell growth and metastasis by embelin through inactivation of metastatic signaling molecules should also be highlighted in light of presented result.

Minor Essential Revisions-

1. In the text and figure legends, embelin concentration is mentioned in mM as opposed to microolar#concentration shown in the figures.
2. A sentence or two (and reference) about the use of Mortalin in creating metastasis should be introduced in the results before further describing the details.
3. In the result section, there seems to be a discrepancy between the text and figure 4C and 4D. The text describes 80% reduction in CFE in metastatic derivative of MDA-MB231, which does not agree with figure 4D.

Typos:

4. In the abstract, page3, line 53, “cancer cells” is written as “cancel cells”.
5. In page 11, line255, the sentence “Fixed cells were ……PBS two twice”, two should be deleted.
6. In page 12, line287, the sentence “The active cleft of…acatalytic” should be a catalytic.
7. In page 16, line378, Figure5B should be Figure5A. Likewise, in line 383, only Fig5C should be mentioned.
8. In the discussion section, page16, line 392, the zinc binding motif, is mentioned as “HexGH……instead of “HExGH……”The active cleft of…acatalytic” should be a catalytic.
9. In the discussion section, page18, line 429, “TNFa” should be “TNFalpha”

Level of interest: An article of importance in its field

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