Reviewer's report

Title: Kalanchoe tubiflora extract inhibits cell proliferation by inducing multipolar spindles, leading to mitotic catastrophe

Version: 1 Date: 12 June 2012

Reviewer: sharon wald krauss

Reviewer's report:

Major Compulsory Revisions

1. Since the major message of this paper is that KT-NB is a promising anti-cancer agent candidate, acting by perturbing accurate mitosis, the authors should present analysis of KT-NB effects in non-cancerous cell lines (if possible, analogous to tumor lines used) to assess if they are relatively more refractory. If they are, this would greatly strengthen this manuscript and the potential for therapeutically attacking cancer cells using KT-NB (and these results should be added to the Abstract). If they are not, then the authors should speculate how KT-NB toxicity may be used to target cancers specifically.

2. The authors concluded that “KT-NB inhibits cell proliferation and reduces cell viability”. How has reduced viability been conclusively demonstrated? For instance, decreased MTT assays could result from decreased metabolism and/or cell death. Before making this claim of reduced viability throughout the text, they should consider whether KT-NB treatment results in non-proliferating but viable inactive cells versus cell killing. If there is ambiguity, this should be acknowledged (and the phrase “and cell death” should be omitted from the Conclusion). This distinction is important although, in either case, KT-NB may be a valuable anti-cancer agent.

3. Discussion, paragraph 3 “Rather than acting on microtubule dynamics, we found that KT-NB disrupts centrosome integrity and induces multipolar spindles”. How has disruption of centrosome integrity been shown? To draw this conclusion, a number of essential centrosome components would have to be tested. In fact, the authors may have some preliminary evidence regarding this point if they revisit their Aurora A- and gamma-tubulin-stained slides and examine centrosome immunofluorescent patterns at interphase cells. Do they mean that multipolar spindles are a hallmark of altered centrosome integrity? Multipolar spindles may result from many disfunctions such as multinucleate cells, cytokinesis failure, centrosome splitting, centrosome amplification etc. In the Conclusion, the phrase “disrupting centrosome integrity” should also be omitted.

4. Discussion, paragraph 5: Modify the statement that the image in Fig 7B shows centrosome clustering. This cannot be concluded without further analysis, eg of centriole numbers. It would be sufficient to say that centrosome clustering can produce bipolar structures.
Minor Essential Revisions

1. Please indicate the units for KT-NB. Micrograms protein?
2. Methods, Reagents, source/composition of MTT solution
3. Fig 3, describe what lines indicate and how their placement was determined
4. Labels are needed for FACS traces in 4A
5. It would be helpful to place a box or shaded area in Fig 4A to indicate the sub-G1 population quantitated in Fig 4C.
6. For Fig 5, state cells used and KT-NB concentration
7. Are data in Fig 7D,E redundant? If so, omit D. If not, please clarify how they differ.
8. Results, KT-NB induces multipolar spindles, paragraph 1: “analyzed by DAPI and microtubule staining”.
9. Discussion, paragraph 5: should use comma after taxol, not semicolon

Discretionary Revisions

1. Conclusion “KT-NB reduced cell viability by exclusively affecting the mitotic cells”. It would be more precise to state “by affecting formation of the proper structure of the mitotic apparatus” or “by perturbing accurate mitosis” or some such phrasing.
2. Background

Paragraph 1: “In animal cells, mitotic spindle assembly is mediated by two pathways.” Modify this sentence with “thus far two major pathways have been identified” or “several pathways mediate” since a number of mechanisms are known. Last paragraph: Since KT is used traditionally in wound healing, I am curious how this is reconciled with the data presented in this manuscript that it inhibits cell proliferation in the wound-healing assay (I cannot access ref 44). Is this due to its antiseptic properties?
3. Results, KT-NB induces multipolar spindles, “Immunofluorescence analysis of #-tubulin and #-tubulin revealed that the microtubule organization and centrosome number were normal in cells treated with KT-NB in interphase (data not shown). Would the authors like to present this data in a Supplementary figure since it has bearing on the genesis of multipolar spindles?
4. Discussion, paragraph 3 It’s a bit risky to strictly conclude that the KT-NB does not affect microtubule dynamics since there could be several activities in the extract with major and minor effects. The authors have wisely stated that this speculation is based on certain features of immunofluorescent images. Since these might not “tell all”, they may want to discuss the possibility of using live cell techniques in the future to assess microtubule dynamics. In this regard, it might also be worthwhile mentioning that live cell imaging could aid in eventually determining the fate of KT-NB treated cells (apoptosis, senescence, necrosis, quiescence). See Major Compulsory Revisions, #2.
Level of interest: An article of importance in its field

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