Reviewer's report

Title: Curcumin potentiates antitumor activity of 5-fluorouracil in a 3D alginate tumor microenvironment of colorectal cancer

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Reviewer: Sankar Sanyal

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Journal: BMC Cancer
Title: Curcumin potentiates antitumor activity of 5-fluorouracil in a 3D alginate tumor microenvironment of colorectal cancer.
Authors: Shakibaei et al.

Comments: The authors described an alginate-based spherical beads culture model of colorectal cancer cells (CRC), HCT-116 and its corresponding isogenic 5-fluorouracil (5-FU) resistant cell lines (HCT-116R). In view of the limitations of carrying out animal based models, the objective had been to provide a 3D-culture model mimicking the tumor microenvironment in vivo in an alginate based scaffold and finding the effects of 5-FU and curcumin in the pathways leading to cell proliferation, invasion, metastasis and adherence properties. The results show that CRC cells encapsulated in alginate were able to proliferate in a 3D colonosphere in an in vivo phenotype and a number of tumor promoting factors such as the chemokine CXCR4, extracellular matrix degrading MMP-9 and the inflammatory transcription factor NF-κB were found to be significantly increased. However, HCT-116R cells over expressed these factors in comparison to the potential HCT-116 cells suggesting an increase in the malignancy behaviour. Also, curcumin potentiated the 5-FU induced decreased capacity for proliferation, invasion and increased more sensitivity to 5-FU of HCT116R cells compared to the HCT116 cells.

It is an excellent paper and deserves to be published in BMC Cancer, as it demonstrates that alginate can provide an ideal tumor environment and curcumin potentiates and chemosensitizes the HCT116 cells to 5-FU based chemotherapy and thus useful in overcoming the drug resistance problem in CRC treatment.

However, the authors may address certain minor points and it may be pointed out that they need not always have to agree with the reviewer's view point.

1. Cell lines and culture: Page 6: How 5-FU resistant cell lines generated and characterised in terms of malignancy. Give references of previous publications.
2. How are the combination doses arrived of 5-FU + curcumin. Is there any preliminary observation.
3. Invasion assay: Page 7: Invasion through matrigel matrix chamber could have been performed (Naksgawa et al. 2005, Carcinogenesis. 26, 1044) for metastasis.
4. Western Blot: Page 8: What is meant by semiquantitative evaluation. Are the values arrived with Image J software and also the values do not seem to be normalised with #-actin.

5. Apoptotic cell death: Page 9: FACS analysis could have been done. For eg. With Annexin V/PI or with apoptotic bleb assay.

6. MTT assay: Page 9: The last sentence “This experiment was repeated 3 times……… etc.” can be deleted. It is already mentioned in the legend to the figure.

7. Results: Page 10: A suitable picture of the colonosphere may be added or if published earlier, the reference may be given.

8. Page 13: Along with CXCR4, MMP-9 and NF-#B, the angiogenic factors could have been studied such as VEGF/VEGFR2, since angiogenesis is an integral part of invasion and metastasis.

9. Page 14: Gelatin zymography of MMP-9 could have been done. Also, beside MMP-9, MMP-2 could have been added.

10. Page 15: Similar cytotoxic profile and apoptosis induced by 5-FU and curcumin in a dose dependent manner (not shown), such data can be put as supplementary.

11. Page 17: Assuming that same number of cells was taken for MTT assay, the OD value given on 14 days is about 4-5 at 550 nm (Fig 2), while in Fig 8 the data is shown as % viable cells and also in parenthesis the absorbance at 550 nm. A note could be given of how the % viable cells arrived or what is the OD value at 100% viable cells.

12. Page 19: Considering the effects of 5-FU and curcumin are not additive but synergistic, what are the mechanisms, alternative or parallel signal transduction pathways. A couple of lines could be written indicating the pathway or molecular crosstalk.

13. Page 21: Certain integral proteins of NF-#B such as IKK and I#B could have been done for their expression as claimed on Page 23, for the phosphorylation of NF-#B.

14. Page 23: Curcumin is shown as chemopreventive agent. But isn’t it more appropriate to describe it as an anti-inflammatory agent.

Level of interest: An article of outstanding merit and interest 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