Reviewer’s report

Title: Cimetidine Inhibits Salivary Gland Tumor Cell Adhesion to Neural Cells and Induces Apoptosis by Blocking NCAM Expression

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Reviewer: Pawan Kumar

Reviewer’s report:

The manuscript by Fukuda and coworkers investigated the role of cimetidine as an anti-tumor agent for salivary gland tumors. Authors have shown that cimetidine can inhibit HSG cell binding to neural cell monolayer as well as inhibit tumor growth in a nude mouse model. The authors have addressed an important aspect of cancer therapeutic, but the study lacks coherent planning. It is difficult to understand the link between different in vitro experiments as well as between in vitro and in vivo experiments. In addition, authors have come to the conclusion that cimetidine inhibits perineural/neural invasion of salivary gland tumors, but there is no data to support that presented in the paper.

Major Compulsory Revisions:

1. In figure 1, it is very difficult to appreciate the % cell adhesion in representative pictures and graphs, e.g. visually there seems to be no difference between cell binding in photograph 1-A (cimetidine 0) and HSG-neural cells (B-right), even though in graphs it is 100% and ~10% respectively. Authors should also present representative photographs for the control groups (-ve control and ICAM-1).

2. In experiments looking at the effect of cimetidine on NCAM and NF-kB gene expression, the authors conclude that NCAM mRNA is induced by the activation of NF-kB. This conclusion is not supported by any data. In the study the authors observe that both NCAM and NF-kB mRNA is upregulated by TNF-a, but there is no evidence presented that NF-kB regulates NCAM. The authors should knock-down NF-kB and then look at the gene expression profile of NCAM.

3. In the tumor development model, the authors have shown that cimetidine inhibits tumor growth and induce apoptosis. As the authors have suggested that cimetidine inhibits NCAM expression in HSG cells, the authors should stain for NCAM expression in tumor samples to confirm that NCAM expression is inhibited in this tumor model. It would greatly add to the data if the authors could add tumor growth time course graph. It will help understand at what point the maximal effect of cimetidine is observed, in vivo.

4. As the authors have looked at the TUNEL staining in vivo, it will add to the data if they could perform the apoptosis assay in vitro by treating the HSG cells with cimetidine at different time points. It will be interesting to look if HSG binding to neural cells inhibits cimetidine mediated apoptosis.
Minor Essential Revisions:

5. In tumor cell binding assay, authors have used semi-confluent neural cell monolayer. What is the rational of using semi-confluent monolayer rather than using confluent monolayer? How do the authors make sure that tumor cells are not binding to the plate (there is no mention of which plate was used)?

Discretionary Revisions:

6. How did the authors distinguish HSG cells from neural cells while counting under phase contrast microscope? It will be much easier to perform this assay and obtain more reliable data by labeling the HSG cells with a fluorescent dye and then quantifying the HSG binding.

7. The authors have also suggested that NCAM regulates TNF-a mediated NF-kB activation in HSG cells (page 12, Fig 4). NCAM is a cell surface adhesion molecule. How does NCAM affect TNF-a mediated NF-kB activation? Does NCAM interfere with TNF-a binding or TNF-a mediated signaling in the HSG cells?

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

'I declare that I have no competing interests'