Reviewer's report

Title: The anticancer activity of lytic peptides is inhibited by heparan sulfate on the surface of the tumor cells

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Reviewer: Ivarne Tersariol

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The text of revised manuscript was improved but the experiments suggested was not done, the revised manuscript does not show directed evidences of involvement of GAGs on the control of the cytotoxic effect of the peptides KW5 and LfcinB. The data are not still sound.

The Major Comments:

1- In order to claim that the effect of 30 mM chlorate treatment was only partial on GAG sulphation the authors had to measure the degree of inhibition promoted by chlorate upon GAGs sulphation for each tumorigenic cells used, since the effect magnitude of chlorate treatment upon GAGs sulphation can vary in according cell type used. The authors did not measure the levels of GAGs sulphation in chlorate experiments! The reference “53” used to corroborate author’s conclusions is not valid for all cell type used in these experiments!

2- The authors did not answer the question “In order to conclude that the difference among IC50 values from MTT curves is due to the presence or not of GAGs in cancer cells. Have the cancer cell lines used in these studies the same molecular mechanisms of cell death preserved?” The authors comment, but they did not prove, that the peptides triggered the same cell death pathway for all cell types used in this study, this is only an assumption.

3- The authors did not answer the following question: “Do soluble CS and heparin inhibit the cell entry of both peptides, at the same rate of inhibition, in pgSA-745 and CHO-K1 cells?”

4- Why serum was not used in the cell death experiments still not clear. The authors said that “serum inhibit the activity of CAPs” in the response for the comments of Oleg Chertov, the absence of serum weakens the cells and stimulate autophagic mechanism in order to survival. So the IC50 values from MTT curves can change in the presence of serum.

5- Why the authors did not measure the amount of [35S]-heparan sulfate released in cellular medium by heparitinase treatments in order to certify that “It is well known that heparitinase is difficult to work with in cell cultures”.

6- Since the peptides KW5 and LfcinB bind to HSPG at the cell surface as the authors proposed in Fig. 10, it is essential to show that these “lytic peptides
(CAPs)” are not endocytosed by these cells in order to affirm that “Our lytic peptides (CAPs) are designed to irreversibly destabilize the cytoplasm membrane whereas cell penetrating peptides (CPPs), which are not used in our study, are designed to penetrate without destabilizing the cytoplasm membrane.” In a previous publication of the group it was showed that these peptides can disrupted the potential of mitochondria membrane and it is observed inside of cells, so how these peptides is penetrating in intracellular compartments?

**Level of interest:** An article of limited interest

**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