Reviewer's report

Title: Ardipusilloside I induces apoptosis via regulating Bcl-2 family proteins in human mucoepidermoid carcinoma Mc3 cells

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Reviewer: Jia-Jun Liu

Reviewer's report:

Review report: Major Compulsory Revisions

General comments:

This manuscript by Xu et al. investigated “Ardipusilloside I induces apoptosis via regulating Bcl-2 family proteins in human mucoepidermoid carcinoma Mc3 cells”. They conclude that “ardipusilloside I could be a new active substance for mucoepidermoid carcinoma, demonstrated the potential mechanism of ardipusilloside I that induce apoptosis by regulating Bcl-2 family proteins level, and suggested a further rationale for development of ardipusilloside I as an anti-cancer agent.”

In this study, few convincing data that helps understand the mechanism of action of ardipusilloside I, and raises the possibility of its use as a therapeutic agent for the treatment of mucoepidermoid carcinoma. The whole field of herbal medicine and the use of ardipusilloside I for the treatment of cancer such as mucoepidermoid carcinoma is generating a lot of interest.

Though this is indeed an interesting reagent that seems to show desirable anticancer effect against mucoepidermoid cancer cell line, there are several things that need to be thought about, especially in terms of therapeutic value. Specificity of action, so that it only induces cell apoptosis or death in the targeted cancer cells, whilst sparing the normal tissues or cells. Generally, for a lead molecule to be considered desirable, sub-micromolar activity is required. And finally, to a lesser extent really, is the detailed mechanism of action of this compound. Apoptosis can be triggered in a number of ways, ultimately leading to an intrinsic pathway, or the extrinsic pathway which directly leads to the activation of the apoptosome and caspase cascades. Drug companies are interested in mechanism to the extent that targeted therapies can offer many advantages, especially in terms of combination therapeutic approaches. A targeted therapy also implies greater control over specificity of action, which would ultimately increase the all-important therapeutic window.

The authors do not take into account the findings regarding the cell biology of these molecules: Bax and Bcl-2 are not only regulated by expression but also by posttranslational modification and several conformational switches. Evidence for involvement of these 2 molecules in cell death by ardipusilloside I is only descriptive and functional evidence is lacking. Experimental data in this paper are scarce and the whole study is, at best, rather preliminary.
Specific recommendations:

1. In the abstract, the authors should declare their objective of this study, or is the word “Background” a typo error? Also, the molecular structure of ardipusilloside I should be provided in the introduction.

2. Only one cancer cell line (Mc3) was used in the study, the authors need to demonstrate that the compound specifically induces apoptosis or cell death in more (two or three) cancer cell lines, but not normal tissues or cells.

3. Some species specific serum esterases can break down small molecules really fast, so try different formulations, serum concentrations, species of serum, or even try replacing serum with a synthetic replacement. Alternatively, the authors might apply the compound to the cells in the absence of serum for some period of time, then replace the serum after the cells have had a chance to absorb it. However, keep in mind the importance of controls when doing these sorts of modifications to experimental design.

4. The MTT assay is used mainly describes the mitochondrial functional capacity alone, as a tool of cell proliferation is not enough. It is important the authors to show data using proliferation markers (either 3TdR incorporation in tumoral cells, or flow cytometric assessment of S-phase fraction based on BrdU labeling, or immunocytochemical staining for Ki-67 or PCNA proteins).

5. In figure 3, the electrophoresis results showed just like “smear” line, and no typical “DNA Ladder” was found. Also, the expression of bax and bcl-2 should be detected before 24 h (before apoptosis occurred).

Minor recommendations:

Though some data of this manuscript is of quality, it could be better if the authors have a profound discussion focusing on their own results.

There are many grammar or typo errors in the manuscript, it is better that the draft has been read and detected by English native speakers before submission.