Reviewer’s report

Title: Estrogen and Soy Isoflavonoids Decrease Sensitivity of Medulloblastoma and Central Nervous System Primitive Neuroectodermal Tumor Cells to Chemotherapeutic Cytotoxicity

Version: 0 Date: 19 Feb 2017

Reviewer: Ivonne Rietjens

Reviewer's report:

The manuscript by Belcher et al. describes the decrease in sensitivity of medulloblastoma and central nervous system primitive neuroectodermal tumor cells to chemotherapeutic cytotoxicity upon coexposure to estrogen and soy isoflavones.

There are some issues the authors should reconsider and modify and these can be summarised as follows.

1) The data as presented do not clearly characterise the effects of the estrogen and isoflavone alone. Since these compounds may increase cell proliferation the effects observed may rather be explained by proliferation of the cells masking the cytotoxicity, and that is not the same as really a decrease in sensitivity. Just two opposite effects. The concentration at low concentration of the chemotherapeutic agents (for example in figure 1A) was set to 100%, masking such a possible effect. For a real adequate conclusion, the actual proliferation in the absence or presence of the estrogens at zero concentration of the chemotherapeutic agents should also be presented. This holds for all figures. Depending on the outcomes the text at many places and even the title of the paper should be reconsidered and modified: If the cells proliferate more in the presence of estrogen that may be the underlying explanation; just two opposite events happening at the same time; Estrogen induced proliferation and drug induced cytotoxicity both with a somewhat different EC50 and this is not the same as a modified sensitivity towards the chemotherapeutic agents. Figure 3 and 4 present data that such proliferation indeed occurs. This implies that all figures should be extended including bars for the situation - and + E2 at zero concentration of the test compound. And that the conclusions and all text on decrease in sensitivity should be modified. Preferably the EC50 of the two effects should be determined: EC50 for proliferation and IC50 for cytotoxicity and then the sum may be the curves now provided. All this is needed to answer the question how the authors can exclude the conclusion that the effect is a reflection of a combination of two separate effects instead of a decrease in sensitivity.

2) Page 6 line 106: add the ER specificity of fulvestrant, is it and antagonist for ERalpha,beta or both?
3) The authors should add data on the actual levels of ERalpha and ERbeta in the cells studied. This is essential for understanding the results observed. For example do the cells express ERalpha? This would provide an explanation for estrogen induced cell proliferation, especially if ERalpha levels dominate over those of ERbeta. This information is essential to fully understand the effects reported.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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