Reviewer's report

Title: Cisplatin-induced caspase activation mediates PTEN cleavage in ovarian cancer cells: a potential mechanism of chemoresistance.

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Reviewer: Barbara Vanderhyden

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The authors have analyzed four ovarian cancer cell lines for their expression of PTEN, phospho-AKT, caspases, and IAPs in response to cisplatin. Cisplatin treatment suppresses levels of PTEN and increases phospho-AKT in only one of the four cell lines (A2780). This cell line also shows upregulation of several cleaved caspases, and decreased abundance of BCL-2. Blocking proteosome degradation has no effect on PTEN levels, but treatment with caspase inhibitors and overexpression of BCL-2 are able to prevent cisplatin-induced reduction in PTEN abundance. The results indicate that BCL-2 and caspases are involved in cisplatin-mediated suppression of PTEN.

The manuscript is generally a good piece of work and implicates PTEN in the response of ovarian cancer cells to cisplatin. The major weaknesses are the lack of correlation with any biological response, and an unclear conclusion related to which conditions in ovarian cancer cells determine whether PTEN is involved in the cellular response. Previous work has shown that cisplatin induces apoptosis by mechanisms that involve suppression of phospho-AKT, so the biological consequences of upregulation of phospho-AKT in these cells are unclear. Some additional information about the cell lines used, their PTEN status and biological response to cisplatin would strengthen this manuscript considerably.

Major Compulsory Revisions:
1. The manuscript needs to be revised to correct the many errors in grammar.
2. The evidence to support the conclusion that cisplatin causes post-translational degradation of PTEN is limited. The results show that mRNA levels are not altered (suggesting that transcription may not be affected) and proteosomal degradation does not play a role, but factors that regulate the translation or stability of the PTEN protein are not investigated. The conclusion in the Abstract should therefore be modified.
3. It is not clear what results in this study suggest a role for PTEN in the development of chemoresistance? Are the effects of cisplatin on PTEN levels irreversible? What is the PTEN status of the ovarian cell lines used in this study - do they all have a normal (i.e. not mutated) PTEN gene? Is anything known about PTEN expression in chemo-sensitive vs. chemoresistant ovarian cancers?
4. The biological consequence of cisplatin treatment of A2780 cells has been
shown in previous studies to be the induction of apoptosis. It is not clear how suppression of PTEN and increased phospho-AKT relates to the viability of these cells in this study, although several experiments are interpreted as if apoptosis is induced, at least to some degree.

5. The relative degree of chemosensitivity of SKOV3 and OVCAR-3 cells (compared to A2780 and A2780CP) is not clear. Do these cells undergo apoptosis in response to the dose and length of exposure to cisplatin used in this study? It is not clear why only one of the four cell lines used shows a suppression in PTEN levels in response to cisplatin treatment.

6. The authors have investigated the intracellular localization of PTEN, but there is no interpretation or discussion of the results.

7. The Results section states that XIAP expression is decreased in OVCAR-3 cells, but this is not evident in the figure (Fig. 6D).

8. The data shown in Figure 7 are an important part of the study. Since the experiment was done three times, the manuscript could be strengthened considerably by providing the quantitative assessment of these data. The interpretation stated in the Results section (that overexpression of BCL-2 could resist cisplatin induced apoptosis....) should be moved to the Discussion section, or stated in the correct context, since apoptosis was not evaluated in this study.

9. Brief mention of the mechanism of action of the caspase inhibitors would be appropriate, since some appear to have an impact on the abundance of cleaved caspase proteins, and others do not.

10. Some of the statements in the Discussion are not supported by the data. There is no experiment that shows proteolytic cleavage of PTEN or PTEN degradation. The mechanism by which PTEN is suppressed by cisplatin remains unclear, so the conclusions in this regard should be revised. The Discussion also states that there is a low endogenous level of BCL-2 in A2780 cells, but this is not supported by the data shown in Figure 6.

Minor Essential Revisions:
1. BGS should be defined.

Level of interest: An article whose findings are important to those with closely related research interests

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

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.