Author’s response to reviews

Title: Breast cancers with high DSS1 expression that potentially maintains BRCA2 stability have poor prognosis in the relapse-free survival

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Author’s response to reviews: see over
Dear Dr. Khalil Helou,

Thank you for your inviting us to revise our manuscript entitled "Breast cancers with high DSS1 expression that potentially maintains BRCA2 stability have poor prognosis in the relapse-free survival" (Manuscript Number: 2055985069102570) written by Andri Rezano et al. In response to reviewer’s comments, we have repeated additional experiments to show the better and sufficient immunoblotting of DSS1 expression in the new Figure S4, and finally prepared the new version that answers all the inquiries raised by your reviewers. Our answers are described in the point-by-point reply sheet. We thank you and your reviewers for understanding our message of the manuscript and providing us important and helpful criticisms regarding the data and our description. We hope that the new version of the manuscript is now suitable for BMC Cancer.

We look forward to hearing from you soon.

Sincerely yours,

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Point-by point replies to the reviewers’ criticisms and suggestions

We thank the reviewers for their important and constructive comments to our manuscript. Here, we are happy to be able to respond to all the questions, criticisms, and suggestions provided from the reviewers. We performed additional experiments, and changed the figures and the description in the Materials and Methods, Results, and Discussion as marked with the blue color ink in the text.

Reviewer #1: Dr. Nicole Dalla Venezia

Reviewer's report:
The authors investigated how the expression of DSS1, a protein that stabilizes BRCA2, is associated with breast cancers. They found that high-DSS1 patients show a poor prognosis. They further investigated using two breast cancer cell lines, the P53-wild type MCF7 and the P53-mutated MDA-MB-231, the impact of DSS1 over-expression or knockdown on cellular proliferation and DNA damage sensitivity. Even though the mechanisms of DDS1-mediated resistance to DNA-damaging drugs are not clear regarding P53 impact, the authors describe several novel findings. The high DSS1 expression could be a marker for drug resistance in breast cancers. From a therapeutic point of view, DSS1 knockdown could serve in combination with DNA-damaging drugs as an anti-tumor drug. In general, the paper is well written. Background, experiments and results are well described. However, some criticisms and questions can be raised concerning the illustrations and discussion, according to the following comments:

1/ Major Compulsory Revisions
a/ The discussion is a very extensive text that largely appears like an extra background and poorly relates to the findings reported. It has to be shortened and focused to highlight the impact of the findings.

--- In response to the reviewer’s comments, we shortened the discussion section substantially to focus on our novel findings concerning the impact of DSS1 in breast cancers.
Authors found in figure 1 that DSS1high group showed a worse prognosis in comparison with the DSS1low group in breast cancer cases with high P53 expression. Because of the crucial role of P53, authors further used two cell lines that present the particularity to contain either wild-type P53 or mutated P53. Surprisingly, results obtained with these cell lines are not discussed regarding the P53 status. See as follow:

- In figure 2, DSS1 overexpression renders only MCF7, but not MDA-MB-231, resistant to treatment with CPT, whereas DSS1 overexpression reduces CPT-induced DNA damage in both MCF7 and MDA-MB-231 cells.

- At the end of “results”, authors indicate that DSS1 depletion increases chemosensitivity in cancer cells containing either wild type or mutant P53. Therefore, results obtained with the two cell lines should be discussed regarding the potential role of P53, at least in “discussion”.

--- According to the suggestion, we described carefully regarding the potential role of DSS1 under various p53 states in the Discussion section.

--- Figure S4B is described by authors as showing “a marked reduction in DSS1 protein”. This cannot be concluded from the western blot displayed in the figure.

--- Since DSS1 is 8-kDa in size and highly acidic, usual SDS-PAGE and western blotting do not work well for the endogenous DSS1 protein. Thus, we repeated experiments by changing multiple conditions and finally obtained the better condition to show the rightness of our description “a marked reduction in DSS1 protein” in the new Figure S4. Our modification of experimental protocol is indicated in Supplementary Methods (Western blot).

2/ Minor Essential Revisions:

a/ Figures S4C and figure 4A-left show almost the same figures, causing the interpretation to be very confusing. It is clear that Figure S4C used Si-DSS1(b) whereas it is not indicated (only in “methods”) that Figure 4A-left used Si-DSS1(a). This point should be elucidated, otherwise it could be tempting to speculate that the apparent effect on cell growth (MTT test) and cell number is not due to DSS1 knockdown.
---We clearly used the term “siDSS1-(a)” in Figure 4 to 6 because siDSS1-(a) was used as a representative throughout this manuscript.

b/ The MTT results showed in Figure S4C should be accompanied by a western blot using Si-DDS1(b) transfected cells, for the same reason as above: results obtained with MTT test may not be correlated with DSS1 knockdown.

---In response to the reviewer’s comment, we also performed anti-DSS1 immunoblotting using siDSS1-(b)-treated cells, which showed the similar change compared with those of siDSS1-(a). The data were added to the new Figure S4B.

c/ In “methods”, the sequence of the SiCtrl is missing. This sequence must be described in that part of the manuscript.

---We added the sequence information of all siRNAs including SiCtrl-(a) and SiCtrl-(b) in the Methods section.

Thank you for your careful reading the manuscript and providing the constructive and helpful comments. Owing to the reviewer’s comments, the manuscript is now polished markedly to describe the message concisely.

Reviewer #2: Dr. Chandra Prakash Prasad

Reviewer's report:
The manuscript entitled 'Breast cancer with high DSS1.....relapse free survival' authored by Rezano et al is a well written article where authors have shown that high DSS1 association with worse prognosis and shorter relapse free survival. Moreover, the study indicates that DSS1 knockdown in combination with chemotherapy can be effective treatment for breast cancer. The sample size for breast cancer (IBC's) cohort in the present article is satisfactory to carry out the statistical analysis. The data is very well presented and supported by in-vitro studies.

Note: There is a spelling mistake for 'Prognosis' everywhere in the article, including the title itself.
--- Thank you for checking the type errors in the manuscript. We used “Prognosis” in the new manuscript.