Author's response to reviews

Title: DKK1 is a potential novel mediator of cisplatin-refractoriness in non-small cell lung cancer cell lines

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Author's response to reviews: see over
Cover letter for MS: 2048730946141488

Dear Editor,

Thank you for the additional comments, we are very grateful for having the possibility to submit our re-revised MS 2048730946141488 by Hogir Salim, Dali Zong, Petra Häåg, Metka Novak, Birgitta Mörk, Rolf Lewensohn, Lovisa Lundholm and Kristina Viktorsson. We have followed the suggestions by the reviewer and changed the manuscript title to “DKK1 is a potential novel mediator of cisplatin-refractoriness in non-small cell lung cancer cell lines”. Please find below our comments to the points raised by reviewer 1 and the references to the manuscript where changes have been introduced.

On behalf of the authors,

Kristina Viktorsson and Lovisa Lundholm

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Responses to reviewers’ questions for MS: 2048730946141488

Reviewer 1: Fredrik Jerhammar

Reviewer's report:
Major compulsory revisions:
As pointed out in the first review, I find the discrepancy of the three microarray replicates an issue. The authors agree that it may be a concern, continuing to hypothesize that it might be due to expansion of different resistant clones. This is, however, not tested. It is an assumption. If more replicates had been done with similar variation, that assumption would have had stronger impact.

Comment: We do agree that this is our interpretation and assumption of the results. Accordingly we have rephrased the sentences within the manuscript dealing with this issue. Thus in the results on p. 12-13 we have made the following deletions (strikethrough) and additions/changes (underlined): Despite similar treatment conditions in the three biological replicates, yet they showed some differences in cisplatin response (Fig.1B), which may hint towards possible heterogeneous mechanisms of refractoriness among the surviving NSCLC clones after cisplatin treatment.
We reasoned that as cisplatin may confer resistance in multiple ways, the heterogeneity among the biological replicates in terms of numbers of genes showing alteration and their difference in magnitude may indeed could possibly reflect a biologically heterogeneous response but it cannot be ruled out that also experimental variations by other means could contribute to the observed results.
In the discussion on p. 21 we have added: “A possible reason may be that few prominent long term effects are seen on the RNA level 9 days after cisplatin treatment. The simplest however, one interpretation of this outcome is that..” as well as “Hence, our interpretation of the data is that we speculate that even small initial variations in cisplatin responsiveness can induce certain clones to become dominant.”

While DKK1 is shown to have statistically significant effects on clonogenic survival after cisplatin treatment (Figure 5D), the statistics behind this result needs to be clarified. What is the p-value of the decrease in clonogenic survival observed with only si1 treatment?

Comment: The clonogenic survival was significantly decreased with only si1 untreated (p=0.003) compared to NT untreated, but not for si2 untreated compared to NT untreated. The viability after cisplatin alone was also significantly decreased for both NT and both siRNAs. For the sake of clarity, we however chose to focus on the difference between si cis versus NT cis in the figure. We have added the following sentence to the figure legend of Figure 5D on p. 38: ***; p < 0.005, **; p < 0.01, when comparing DKK1 siRNA-transfected to NT siRNA-transfected cells after cisplatin treatment.

Minor essential revision
Title: Only cell lines have been tested. The title suggests that tumor samples have been tested. Also, protein levels of Dickkopf-1 is not addressed at all, should be changed to DKK1. "DKK1 is a potential mediator of non small cell lung cancer cell lines" is a title more in concordance with the major results.
Comment: The title has been updated to: DKK1 is a potential novel mediator of cisplatin-refractoriness in non-small cell lung cancer cell lines. As recommended by the reviewer, Dickkopf-1 has been changed to DKK1 and “…cell lines” added to stress that the work was not carried out in vivo.