Title: Targeting cell migration and the endoplasmic reticulum stress response with calmodulin antagonists: a clinically tested small molecule phenocopy of SEC62 gene silencing in human tumor cells

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

again I would like to thank you for your positive evaluation of our manuscript, titled “Targeting cell migration and the endoplasmic reticulum stress response with calmodulin antagonists: A clinically tested small molecule phenocopy of SEC62 gene silencing in human tumor cells”, and for giving us the opportunity to deal with the encouraging comments of the reviewers as well as to submit a revised version of our manuscript. As recommended by the editorial office we used the assistance of the Edanz editorial service to improve the quality of the written English and also to make sure that the manuscript conforms the journal style of BMC cancer. In addition, I again want to provide you our point by point response to the reviewers concerns already included in our last revised version. The main points of this last revision were the addition of measurements of ER-lumenal calcium and quantitative data for our migration assays, and the incorporation of all supplemental figures into the main manuscript.

Response to reviewer 1’s suggestions (Alexei Tepikin):

1. The reviewer suggested that it would be advantageous to directly measure ER-lumenal Ca\(^{2+}\) after treatment with calmodulin antagonists and also to investigate the influence of such a treatment on store operated calcium entry (SOCE). To deal with this point we added the new figures 2B and 2C. In figure 2B we used HeLa-CES2 cells, which contain an ER lumenal carboxyl esterase and allow efficient dye loading of the ER to directly visualize the decrease in ER lumenal Ca\(^{2+}\) concentration. These experiments showed a significantly faster Ca\(^{2+}\) efflux from the ER after treatment with ophiobolin A or TFP compared to the solvent control, thereby, proving our conclusion, that ER- Ca\(^{2+}\) efflux is responsible for the increase in cytosolic Ca\(^{2+}\) concentration under these conditions. In figure 2C we show the significantly increased cytosolic Ca\(^{2+}\) after treatment with calmodulin antagonists and extracellular Ca\(^{2+}\) addition. This effect could be inhibited by depletion of Sec61\(\alpha\) indicating the relevance of the Sec61-complex for SOCE under these conditions.

2. We apologize for this mistake and corrected the Fura-2AM concentration to 4 µM.

3. As recommended, we made our statement clearer by adding “…binding of calmodulin to the Sec61-complex”.

4. As recommended, we changed the sentence as follows to specify our intention: “Also sensitivity to thapsigargin (Figure 3D) and thapsigargin induced Ca\(^{2+}\) leakage from the ER were increased after SEC62\(_{D308A}\) expression”.

5. We moderated our conclusion as recommended by the reviewer and also restructured the sentences to state clearly on the molecular mechanism
of the resistance of Sec62 overproducing tumor cells to treatment with thapsigargin.

6. As recommended by the reviewer, we moved the supplementary Figure S1 to the new Figure 5A, the quantitative data to the migration assays from supplementary Figure S2 to the new Figure 4D and we moved our functional model from the supplementary Figures S3 and S4 to the new Figure 7.

Response to reviewer 2’s suggestions (Kasper Rouschop):

1. We carefully proofread the entire manuscript to improve the language and especially focused on the sentences mentioned by the reviewer.
2. We apologize for our mistake. As supposed by the reviewer indeed the median was used to split the patient cohort and not the mean value. We corrected that in the Methods part.
3. As recommended by the reviewer we added quantitative data to the migration assays shown in Figure 5B and C. For the pictures shown in Figure 6C the quantitative data was already given in Figure 6D and we also added the quantitative data for the pictures in Figure 4D to the micrographs and added quantifications to Figure 3C.
4. We replaced the Western blot shown in Figure 3B by the blot from another experiment which is also included in the data shown in Figure 3C.
5. As reviewer 1 recommended moving all supplementary figures to the main manuscript we decided to completely waive the supplement. Therefore we also left the data from Figure 4 in the manuscript, but due to the rearrangement of the figures, the new Figure 4 now also contains the migration data after titration of the calmodulin antagonists and now no longer contains only the negative data mentioned by the reviewer.

We hope that our alterations meet the reviewers’ criticisms to their satisfaction and that the manuscript now meets the criteria for publication in the BMC Cancer.

Yours sincerely,

Markus Greiner