



Reply to: Target expression is a relevant factor in synthetic lethal screens

Yosi Gilad¹, Yossi Eliaz², Yang Yu¹, Adam M. Dean¹, San Jung Han¹, Li Qin¹, Bert W. O'Malley¹  [✉] & David M. Lonard¹  [✉]

REPLYING TO I.J. Schultz & H.J.T. Coelingh Bennink *Commun. Biol.* <https://doi.org/10.1038/s42003-022-03746-6> (2022)

The major concern that the authors have risen is the enhanced sensitivity of BC cell lines to SI-12 treatment in our study as a result of the silencing of four genes—*OR4D6*, *OR511*, *NDNF*, and *S1PR1*—in spite of the lack of their expression in these cell lines, according to public databases and previously published studies.

There are a few points that might explain this contradiction:

- It is important to mention that in the studies by Weber et al.¹ and Masjedi et al.², that the authors have mentioned, the transcriptome analysis was performed on untreated samples, while the importance of *OR511* and *OR4D6* in the context of our study manifested under SI-12 treatment.
- We have performed qPCR experiments to access the mRNA levels following siRNA treatment in MCF7 cells. We would like to mention that the apparent lack of concordance between our RT-qPCR data and DepMap RNA-Seq data could be due to false-negative discovery, which is a common issue of RNA-Seq^{3,4}, and that RT-qPCR is capable of greater sensitivity⁵. Moreover, it is well known that many key driver genes, including signaling receptors, are frequently expressed at very low levels in cancer cells^{6,7} and therefore are even more prone for false-negative discovery by RNA-Seq.
- There are evidences that the expression of OR varies across individuals⁸; therefore it might be also the case across cell line subsets.
- It is an unfortunate, but inevitable nature of cancer cell lines that have the same parental origin, but which had been handled differently (in the case of MCF7 cells - for decades), to have a non-homogenous gene-expression profile. In the context of the discussed four genes, we found that it might be best exemplified by the fact that while the expression of *S1PR1* is absent in MCF7 cells, as you have found by interrogation of publically available databases, this gene was found to be expressed in MCF7 cells elsewhere. Furthermore, it was found to have functional importance in this cell line^{9–12}.

Importantly, since the main goal our study was to find gene targets that can be pharmacologically inhibited, the focus of our

study was made on screening candidates that could be validated not only by siRNA perturbation, but also by pharmacological inhibition. However, the authors of this MA focused on four genes, three of which could not be pharmacologically validated (*OR4D6*, *OR511*, and *NDNF*, since, to the best of our knowledge, there are no available pharmacological inhibitors for these three genes). Therefore, we made a clear statement in our paper that these genes are potential anti-cancer therapeutic targets in combination with SI-12 rather than validated ones. The only target out of the four genes that are in the focus of the discussion of this MA that has been validated both by an individual siRNA perturbation and pharmacological inhibition in combination with SI-12 is *S1PR1*. Indeed, our results show that the pharmacological inhibition of *S1PR1* did not contribute to the cancer cell-killing effect in combination with SI-12 (which might support the author's concern regarding a robust and biologically significant expression of this gene). Therefore, this target was not included in our conclusive remarks as a valid target for combination treatment with SI-12. We would like to mention that the lack of complete agreement between initial screening candidates and later individual validations is almost a matter of fact in these types of experiments, and generally, this is why validation experiments are carried out following screening.

Received: 3 August 2021; Accepted: 21 July 2022;

Published online: 19 August 2022

References

1. Weber, L. et al. Olfactory receptors as biomarkers in human breast carcinoma tissues. *Front. Oncol.* **8**, 33 (2018).
2. Masjedi, S., Zwiebel, L. J. & Giorgio, T. D. Olfactory receptor gene abundance in invasive breast carcinoma. *Sci. Rep.* **9**, 13736 (2019).
3. Corley, S. M., MacKenzie, K. L., Beverdam, A., Roddam, L. F. & Wilkins, M. R. Differentially expressed genes from RNA-Seq and functional enrichment results are affected by the choice of single-end versus paired-end reads and stranded versus non-stranded protocols. *BMC Genom.* **18**, 399 (2017).
4. Pomaznoy, M., Sethi, A., Greenbaum, J. & Peters, B. Identifying inaccuracies in gene expression estimates from unstranded RNA-seq data. *Sci. Rep.* **9**, 16342 (2019).
5. Corchete, L. A. et al. Systematic comparison and assessment of RNA-seq procedures for gene expression quantitative analysis. *Sci. Rep.* **10**, 19737 (2020).

¹Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA. ²Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. ✉email: berto@bcm.edu; dlonard@bcm.edu

6. Jordan, J. J. et al. Low-level p53 expression changes transactivation rules and reveals superactivating sequences. *Proc. Natl Acad. Sci. USA* **109**, 14387–14392 (2012).
7. Tomita, S. et al. Estrogen receptor alpha gene ESR1 amplification may predict endocrine therapy responsiveness in breast cancer patients. *Cancer Sci.* **100**, 1012–1017 (2009).
8. Verbeurgt, C. et al. Profiling of olfactory receptor gene expression in whole human olfactory mucosa. *PLoS ONE* **9**, e96333 (2014).
9. Lei, F. J. et al. Survival benefit of sphingosin-1-phosphate and receptors expressions in breast cancer patients. *Cancer Med.* **7**, 3743–3754 (2018).
10. Liu, S. et al. S1PR1 regulates the switch of two angiogenic modes by VE-cadherin phosphorylation in breast cancer. *Cell Death Dis.* **10**, 200 (2019).
11. Nagahashi, M. et al. Targeting the SphK1/S1P/S1PR1 axis that links obesity, chronic inflammation, and breast cancer metastasis. *Cancer Res.* **78**, 1713–1725 (2018).
12. Zhou, P. et al. MicroRNA-363-mediated downregulation of S1PR1 suppresses the proliferation of hepatocellular carcinoma cells. *Cell Signal* **26**, 1347–1354 (2014).

Author contributions

Y.G. and D.M.L. wrote the response to matters arising. Y.G., Y.E., Y.Y., A.M.D., S.J.H., L.Q., B.W.O., and D.M.L. have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Bert W. O'Malley or David M. Lonard.

Peer review information *Communications Biology* thanks the anonymous reviewers for their contribution to the peer review of this work. Primary Handling Editor: Eve Rogers.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2022