Neuro-Oncology
22(5), 734–735, 2020 | doi:10.1093/neuonc/noaa037 | Advance Access date 27 March 2020

Reply to Letter to the Editor

Reply to comment on "GRP94 promotes brain metastasis by engaging pro-survival autophagy"

We thank Yoshida[1] for his interest in our recently published paper by Santana-Codina et al.[2] We appreciate his positive feedback and valuable comments relative to our study on the role of glucose-regulated protein 94 (GRP94) in brain metastases (BM). Here, we describe a metabolic switch consisting of activation of the unfolded protein response and pro-survival autophagy to relieve metabolic stress in low glucose conditions, facilitating BM cell survival.[2]

Yoshida[1] emphasizes the importance of evaluating p62/SQSTM1 in metastatic tumor cells located in the tumors' invasive front and perinecrotic regions. We agree that p62 levels could help clarify if there is increased or impaired autophagic flux in hypoxia-inducible factor α–positive cells. BM cells show strong dependence on autophagy, and in fact inhibiting autophagic flux with chloroquine impaired BM development. Despite the well-known dual role of autophagy,[3] we show that, in our model, autophagy is a pro-survival mechanism mediating adaptation to low nutrient conditions. Since cells expressing LC3B are close to necrotic areas in BM, it is tempting to speculate that autophagy might also be implicated in sheltering subpopulations in the hypoxic microenvironment.

Yoshida references edelfosine, a new therapy in phase II clinical trials as a promoter of autophagic cell death in triple negative breast cancer (TNBC). Edelfosine is an apoptotic inducer in cancer cells by promoting mitochondrial membrane permeability independent of reactive oxygen species, and lipid rafts redistribution from the plasma membrane to mitochondria.[4] The mechanisms by which edelfosine induces autophagic cell death or apoptosis are complex and likely dose dependent.[5] Moreover, autophagy has a dynamic role that can either suppress or promote tumor growth at different stages of tumor progression. In fact, our data are in accordance with previous studies showing a critical temporal dependence on autophagy, especially at initial stages of the metastatic establishment, where autophagy would promote the outgrowth of dormant metastatic cells.[6] We agree with Yoshida that targeting brain adaptive cancer stem cells (CSCs) is a promising field to prevent or treat TNBC-BM, either with edelfosine or with other autophagy preventive drugs.

Finally, we agree with Yoshida on the importance of evaluating the effect of GRP94 on mitophagy in brain CSCs. In this regard, we have shown that BM cells present elongated mitochondria,[7] a phenotype observed in response to starvation and autophagy induction in order to maintain ATP production and cell viability.[8] We showed that BM cells exert a high ability to oxidize fatty acid and compensate hypoglycemic stress due to an overexpression of proteins involved in fatty acid synthesis and degradation, whereas GRP94 ablation restored glucose dependence and decreased tumorigenicity in vivo.[7] Activation of autophagy (and likely mitophagy) might be concurrent events under starvation conditions to maintain energy production and mitochondrial integrity. Further studies are required to understand the interplay between mitophagy, bulk autophagy, and metabolic dependencies in BM cells.

In conclusion, we appreciate Yoshida's comments on our work. We agree that continued research on GRP94 and its modulation of mitophagy and metabolic adaptation is a promising and exciting field that may lead to new therapeutic opportunities in BM.

Funding

This study was supported by grants from the Spanish Ministry of Health and Consumer Affairs, FIS-PI14/00336 and FIS-PI18/00916, from the I+D+I National Plan with the financial support from ISCIII-Subdirección General de Evaluación and the Fondo Europeo de Desarrollo Regional (FEDER), and by grants from Generalitat de Catalunya 2017_SGR_1305.

Conflict of interest statement A.S. is inventor of the patent US 9,645,150 B2 and EP 2440931 named "Methods for determining the risk for developing brain metastasis, and a kit to carry out said method", which refers to GRP94's potential as biomarker to predict brain metastasis and to target therapies. All other authors have declared that no conflicts of interest exist.

Naiara Santana-Codina, Josep Gonzalez, and Angels Sierra

Biological Clues of the Invasive and Metastatic Phenotype Group, Bellvitge Biomedical Research Institute-IDIBELL, Hospitalet de Llobregat, Barcelona, Spain (N.S.C., A.S.); Autonomous University of Barcelona, Campus Bellaterra, Cerdanyola del Vallés, Barcelona, Spain (N.S.C.); Department

of Neurosurgery, Hospital Clinic Barcelona, August Pi i Sunyer Biomedical Research Institute-IDIBAPS Advances in Neurosurgery Research Group, Barcelona, Spain (J.G.); Laboratory of Molecular and Translational Oncology, Cellex Biomedical Research Center-IDIBAPS, Barcelona, Spain (A.S.)

Corresponding Author: A. Sierra, Laboratory of Molecular and Translational Oncology, Centre de Recerca Biomèdica CELLEX-CRBC- Planta 1ª, Institut d'Investigacions Biomèdiques August Pi i Sunyer-IDIBAPS, Casanova,143, 08036 Barcelona – Spain (masierra@clinic.cat; asierrajim@gmail.com)

References

1. Yoshida G. Comment on "GRP94 promotes brain metastasis by engaging pro-survival autophagy." *Neuro Oncol*. 2020;22(5):732–733.
2. Santana-Codina N, et al. GRP94 promotes brain metastasis by engaging pro-survival autophagy. *Neuro Oncol*. 2020;22(5):652–664.
3. Santana-Codina N, Mancias JD, Kimmelman AC. The role of autophagy in cancer. *Annu Rev Cancer Biol*. 2017;1:19–39.
4. Mollinedo F, Fernández M, Hornillos V, et al. Involvement of lipid rafts in the localization and dysfunction effect of the antitumor ether phospholipid edelfosine in mitochondria. *Cell Death Dis*. 2011;2:e158.
5. Lim SC, Parajuli KR, Han SI. The alkyllysophospholipid edelfosine enhances TRAIL-mediated apoptosis in gastric cancer cells through death receptor 5 and the mitochondrial pathway. *Tumour Biol*. 2016;37(5):6205–6216.
6. Vera-Ramirez L, Vodnala SK, Nini R, Hunter KW, Green JE. Autophagy promotes the survival of dormant breast cancer cells and metastatic tumour recurrence. *Nat Commun*. 2018;9(1):1944.
7. Santana-Codina N, et al. GRP94 is involved in the lipid phenotype of brain metastatic cells. *Int J Mol Sci*. 2019;20:3883.
8. Gomes LC, Di Benedetto G, Scorrano L. During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. *Nat Cell Biol*. 2011;13(5):589–598.