LETTER

REPLY TO YOSHIDA:

Delineating critical roles of MDA-9 in protective autophagy-mediated anoikis resistance in human glioma stem cells

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We appreciate the interest, positive feedback, and useful insights of Yoshida (1) relative to our study by Talukdar et al. (2). We acknowledge the constructive analysis of our work and the valuable comments related to our unique observations on the role of melanoma differentiation associated gene-9 (MDA-9)/Syntenin, a gene initially cloned in our laboratory, in glioma stem cells (GSCs).

Yoshida (1) emphasizes the importance of evaluating mitophagy, selective autophagy-dependent degradation of dysfunctional mitochondria generating reactive oxygen species (ROS), in mediating autophagy in GSCs. We agree that mitophagy is another important area worthy of exploration in the context of the phenomenon we studied in GSCs (1, 2). Defining how the PINK1/Parkin/PI3K axis affects anoikis resistance in cancer stem cells (CSCs) and how ROS might regulate this process is important. We demonstrated previously that the MDA-9/Syntenin and phosphoinositide 3-kinase (PI3K) pathways are interconnected (3) and that MDA-9/Syntenin is central in regulating PI3K (4). Although speculative, MDA-9/Syntenin might also regulate PINK1 and mitophagy through Akt (5, 6). PI3K is important in autophagic regulation (7), which, in turn, regulates anoikis resistance in GSCs. Scrutinizing the effect of mitophagy on anoikis resistance in GSCs may uncover unique mechanistic insights.

Accumulating evidence suggests that mitophagy in CSCs promotes therapeutic resistance, partly by reducing cytotoxic ROS (1). We showed previously that MDA-9/Syntenin regulated Notch and STAT3 activity in GSCs (8) and that Notch also affected PI3K/Akt signaling (9). STAT3 has been reported to regulate ROS (10) and GSC stemness (8), so it will be important to define the interplay between these processes in GSCs. MDA-9/Syntenin might also regulate ROS through STAT3 and BCL-2.

Mitophagy can be studied by observing mitochondria in the autophagosomes/autolysosomes with transmission electron microscopy and fluorescent double-staining using GFP-LC3 and MitoTracker Red in live cells. We have performed GFP-LC3 studies to detect autophagy and studies using MitoTracker and GFP-LC3 to detect mitophagy would be of value. Evaluation of outer mitochondrial membrane-associated proteins is also worth investigating as a consequence of protective autophagy and anoikis resistance in GSCs.

IFN-γ–regulated autophagic cell death (ACD), especially through involvement of PI3K or caspases, is an intriguing possibility (1). In addition to our PI3K studies, in our earlier experiments, we found that MDA-9/Syntenin regulated GSC survival and that its loss promoted caspase activation (8). Accordingly, these observations help to illuminate the role of MDA-9/Syntenin in protective autophagy and anoikis resistance in GSCs. In our study focusing on GSCs and anoikis resistance (2), we emphasized macroautophagy-related cell death. However, studying alternative autophagy pathways and how autophagy might be connected to other signaling pathways is an area of significant interest worthy of continued scrutiny.

To conclude, we appreciate that Yoshida (1) considers our work elegant and relevant to mechanistically linking autophagic regulation to anoikis resistance in CSCs. We agree that continued research on MDA-9/Syntenin offers promise for developing therapeutic small-molecule inhibitors (11) and virus vectors to obstruct the ability of

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this key regulatory molecule to promote cancer invasion and metastasis. Continued research is necessary to define further which type of ACD occurs in GSCs following genetic and pharmacological inhibition of MDA-9/Syntenin.

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