LETTER

Molecular machinery underlying the autophagic regulation by MDA-9/Syntenin leading to anoikis resistance of tumor cells

Go J. Yoshida[a,1]

I read with great interest the excellent work of Talukdar et al. (1), in which the authors demonstrate that melanoma differentiation associated gene-9 (MDA-9)/Syntenin (syndecan binding protein; SDCBP) regulates protective autophagy in glioblastoma stem cells (GSCs) through two cascades: First, the complex composed of MDA-9/Syntenin and focal adhesion kinase (FAK) increases phosphorylated Bcl-2 via PKCα, and, second, EGFR activated by the complex of MDA-9/Syntenin and FAK induces autophagy-related molecules such as Atg5, LC-3, and Lamp1 (1). Anoikis resistance is one of the hallmarks of cancer, which is essential for circulating tumor cells to persistently survive in the bloodstream and to exhibit the colonization/proliferation in the premetastatic niche (the favorable microenvironment for undifferentiated tumor-initiating cells to potentially form the distant metastatic disease) (2).

Although shRNA-mediated depletion of MDA-9/Syntenin exhibited an increased number of autophagosomes regardless of *EGFR* mutations in GSCs (1), it seems to be important to evaluate mitophagy, selective autophagy-dependent degradation of dysfunctional mitochondria generating reactive oxygen species (ROS). Mitophagy is mainly regulated by the PINK1/Parkin axis. Accumulated PINK1 on the outer mitochondrial membrane (OMM) of depolarized mitochondria results in impaired PARL-mediated PINK1 cleavage, which leads to the recruitment of Parkin. Mitochondria-localized Parkin promotes ubiquitination of OMM-associated proteins, which causes binding with p62/SQSTM1 (3, 4). Moreover, Parkin can also interact with Ambra1, which in turn activates PI3K to facilitate mitophagy (4). Remarkably, mounting evidence suggests that mitophagy in cancer stem-like cells (CSCs) brings about therapeutic resistance partly due to reduced cytotoxic ROS (5, 6). There are several methods to evaluate mitophagy, including observation of mitochondria in the autophagosomes/autolysosomes with transmission electron microscopy and fluorescent double staining using GFP-LC3 and MitoTracker Red in live cells. Notably, Western blotting analysis of OMM-associated proteins such as Tom20 and Mitofusin is likely to cause misinterpretation. After all, they are preferentially degraded by the proteasome/lysosome cascade.

I do agree with the authors' description that Atg5 dysregulation contributes to autophagic cell death (ACD) depending on the cell context (1, 7). An increased number of autophagic vacuoles are accompanied by cell death after treatment with interferon-γ (IFN-γ). This type of ACD triggered by IFN-γ can be inhibited by either 3-Methyladenine (PI3K inhibitor) or Z-VAD-FMK (pan-caspase inhibitor) (7). Shimizu and coworkers (8, 9) clarified the molecular mechanism of Atg5-independent alternative autophagy, and they reported that JNK signal is activated in the process of ACD of murine embryonic fibroblasts (MEFs) established from *Bax/Bak* double-knockout (DKO) mice treated with etoposide (VP-16), the chemical of which causes genotoxic stress (8, 9). Notably, DKO-MEFs exhibit ACD independent of caspase, which is strikingly different from the above-mentioned ACD (1, 7).

To conclude, Talukdar et al.'s elegant work (1) elucidated that fine-tuning autophagic regulation is essential for anoikis resistance in CSCs, given that this well evolutionally conserved self-eating phenomenon functions as a "double-edged sword" for tumor cells (6, 10). Inhibition of FAK leads not only to the attenuated phosphorylation level of EGFR but also to the significant up-regulation of autophagy-related molecules to cause cell death with excessive autophagy in GSCs (1). Surely, this research holds much therapeutic promise in terms of the development of small-molecule inhibitors and adeno-associated virus vectors to inhibit MDA-9/Syntenin. However, as of this writing, further investigations are warranted into which type of ACD occurred in the absence of MDA-9/Syntenin and whether the protective autophagy in GSCs is the same as basal autophagy independent of nutrient microenvironment such as starvation.

[a]Department of Pathological Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

Author contributions: G.J.Y. designed research, performed research, contributed new reagents/analytic tools, analyzed data, and wrote the paper.

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[1]Email: medical21go@yahoo.co.jp.

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