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Letter to the Editor

The Role of Valosin-containing Protein in Organelle-specific Autophagy

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Organelle-specific autophagy is a subtype of selective autophagy that delivers damaged organelles for lysosomal degradation, maintaining cellular homeostasis.[1,2] The dysfunction of multiple organelle-autophagy is critically involved in the pathogenesis and progression of various diseases. Furthermore, mitophagy deficiency is linked to various diseases, such as aging, age-related neurodegeneration, and cancer. In late-onset sporadic Alzheimer's disease, it has been shown that the mitochondrial autophagy pathway is impaired, resulting in amyloid beta and tau protein accumulation, and increased oxidative damage and cellular energy deficits.[3] Furthermore, it was found that the mitophagy deficiency induced by Inauhzin, which is a potent surtulin-1 inhibitor, dramatically promotes colorectal cancer cell death through mitochondrial Ca^{2+} overload.[4] In terms of the endoplasmic reticulum (ER), the dysfunction of ER-phagy resulted in a consistent ER expansion, and subsequently, cell death.[5] The deficiency in the autophagy-lysosomal pathway can lead to protein aggregation and the accumulation of dysfunctional organelles, which are signs of Alzheimer's, Parkinson's, Huntington's and prion diseases.[6] Therefore, uncovering the mechanisms underlying the different types of organelle-specific autophagy, and its roles in various conditions would be beneficial to drug development.

Different from the macroautophagy driven by nutrients or energy limitation, selective autophagy recruits the autophagic machinery to the site of the damaged organelles via autophagy receptors, forming autophagosomes, and subsequently fusing with lysosomes for degradation. To date, organelle-specific autophagy includes mitophagy, pexophagy, ER-phagy, ribophagy, lysophagy and nucleophagy.[1] Valosin-containing protein (VCP) is an adenosine triphosphate-driven chaperone that regulates ubiquitinated or unfolded client proteins for recycling or degradation. Upon ER stress, VCP binds with cofactors ubiquitin fusion degradation protein 1 (UFD1) and nuclear protein localization homolog 4 to facilitate ER-associated degradation.[7] An increasing body of studies has revealed that VCP participates in the degradation of several organelles, such as lysosome,[8] ER[5] and mitochondria,[9] as well as in cancer metastasis.[10]

A recent study conducted by Lu *et al*.[9] demonstrated that in Parkin-mediated mitophagy, VCP was recruited to damaged mitochondria with the help of WD repeat domain phosphoinositide-interacting protein 2 (WIPI2), which is an autophagy-associated protein that recruits the ATG12-ATG5-ATG16L1 complex after activation by phosphatidylinositol 3-phosphate (PtdIns3P). Based on a classical mitophagy cell model of HeLa cells that stably expressed the parkin RBR E3 ubiquitin protein ligase (PRKN)-yellow fluorescent protein (YPH cells), the authors used mitochondrial uncoupler carbonyl cyanide chlorophenylhydrazone (CCCP) to induce mitochondrial depolarization in these cells. It was observed that WIPI2 interacted with VCP, and was recruited to the mitochondria after CCCP treatment, suggesting that WIPI2 and VCP play critical roles in mitophagy.

VCP has four domains: N, D1, D2 and C-terminus. Lu *et al*.[9] reported that the N and D2 domains are essential for the interaction of VCP with WIPI2. Furthermore, they observed that two cofactors of VCP, namely, nuclear protein localization protein 4 (NPLOC4) and UFD1, bound with WIPI2, suggesting that WIPI2 interacts with the VCP-NPLOC4-UFD1 complex during mitophagy. In addition, they employed NMS-873, a potent and specific inhibitor of VCP, and observed that the treatment dramatically impaired the mitochondrial recruitment of VCP and prevented outer mitochondrial membrane (OMM) proteins, such as translocase of outer mitochondrial membrane 20 (TOMM20), through mitochondrial depolarization degradation. However, NMS-873 did not prevent the mitochondrial translocation of WIPI2. In addition, it was observed that the MG132 proteasome inhibitor blocked the degradation of OMM proteins, similar to mitofusin (MFN1)-1, MFN2 and TOMM20, in mitophagy induction, suggesting that the mitochondrial recruitment of VCP is critical for the turnover of OMM proteins, in response to mitochondrial damage. Furthermore, such recruitment dramatically decreased in WIPI2 knockout (WIPI2 KO) cells, when compared to wild-type (WT) cells. Importantly, the mitochondrial recruitment of VCP was highly reliant on PINK1 and PRKN (Fig. 1).

Since WIPI2 requires PtdIns3P to achieve its subcellular location, the authors used an effective and selective phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3) VPS34 inhibitor, SAR405,

Abbreviations: CCCP, carbonyl cyanide chlorophenylhydrazone; ER, endoplasmic reticulum; Gal3, galectin-3; Gal8, galectin-8; NPLOC4, nuclear protein localization protein 4; UFD1, ubiquitin fusion degradation protein 1; VCP, valosin-containing protein; WIPI2, WD repeat domain phosphoinositide-interacting protein 2; WIPI2 KO, WIPI2 knockout; WT, wild type.

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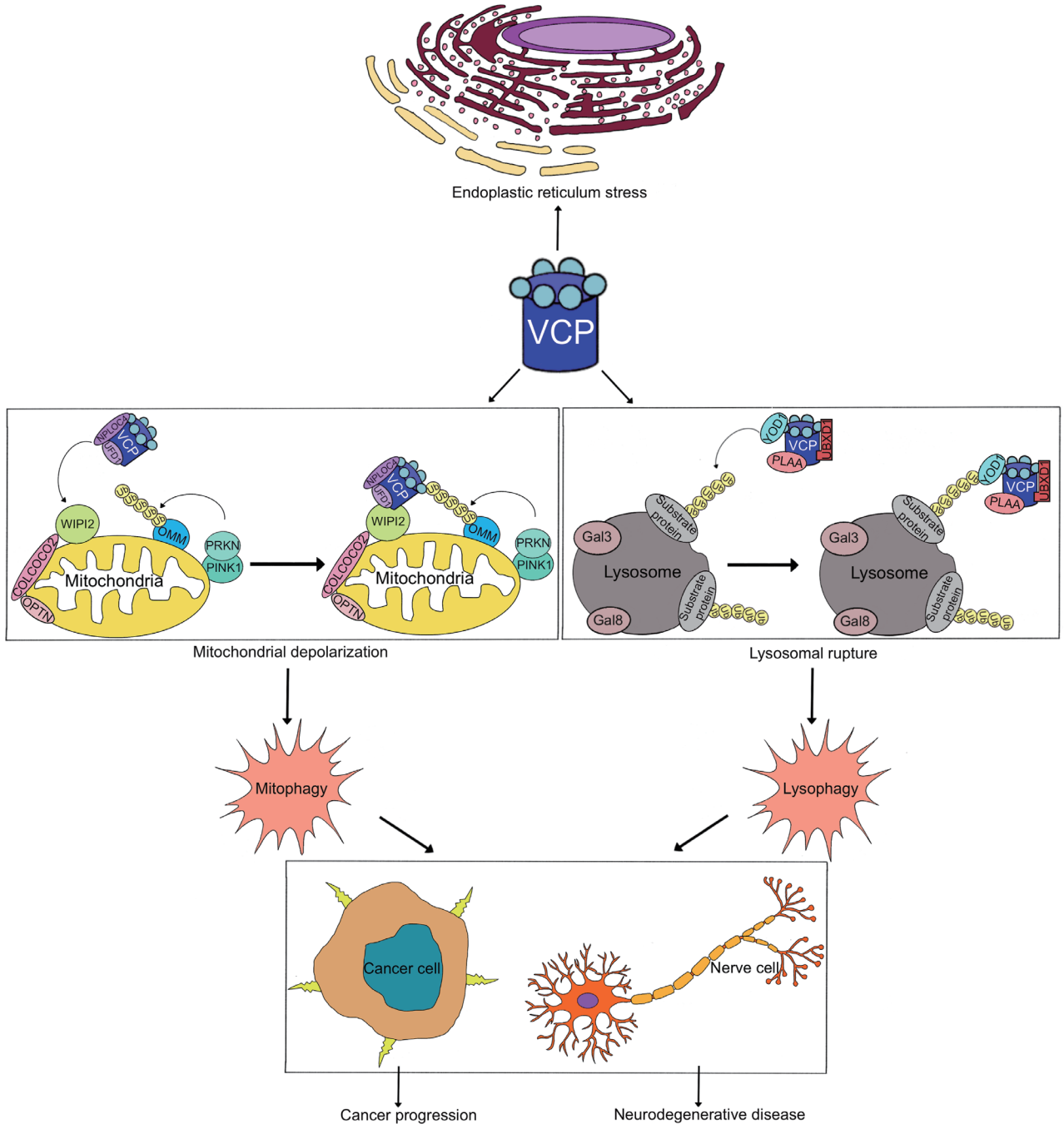

Fig. 1. Scheme of VCP in mediating organelle-specific autophagy. Upon PINK1-PRNK mediated mitophagy, VCP and its cofactors, UFD1 and NPLOC4, recruit to damaged mitochondria by WIPI2, in order to promote mitochondrial clearance. In response to the lysosomal rupture, Gal3 and Gal8 influx to the inner lysosomal membrane and recruit the formation of ubiquitin chains. VCP and its cofactors, UBX domain protein 1, phospholipase A2 activating protein, and YOD1 deubiquitinase, recruit to the damaged lysosomes modified with the K48-linked ubiquitin chain, in order to promote lysophagy. In addition, VCP exhibits a classic function in ER-associated degradation under ER stress. The dysfunction of VCP-mediated organelle-autophagy is critically involved in the pathogenesis and progression of various diseases. ER, endoplasmic reticulum; Gal3, galectin-3; Gal8, galectin-8.

in order to examine the inhibition effects of PIK3C3/VPS34 on the mitochondrial recruitment of VCP. Consistently, the SAR405 treatment inhibited the mitochondrial recruitment of VCP without affecting the total protein level, demonstrating the importance of WIPI2 in the mitochondrial recruitment of VCP upon mitochondrial damage.

It remains unclear whether the cell death induced by mitochondrial depolarization was attenuated in WIPI2 KO cells. The authors found that the subcellular distribution of cytochrome c, somatic (CYCS) was released from the mitochondria in depolarized cells. Furthermore, CCCP induced the release of CYCS from the mito-

chondria to the cytosol in WT cells, but not in WIPI2 KO cells. This was confirmed by the cleavage of caspase-3 and poly (ADP-ribose) polymerase. Interestingly, the WIPI2 KO cells were much more resistant to CCCP or oligomycin-antimycin-mediated cell death, when compared to YPH WT cells, suggesting that WIPI2 deficiency makes cells more resistant to apoptotic cell death through mitochondrial damaging agents.

VCP plays a critical role in mediating lysophagy, the clearance of damaged lysosomes by autophagy. Damaged lysosomes are sensed through the cytosolic binding of galectin-3 (Gal3) or galectin-8 (Gal8) at the inner lysosomal membrane, which recruits the formation of ubiquitin chains. With the help of cofactors, including UBX domain protein 1, phospholipase A2 activating protein, and YOD1 deubiquitinase, ubiquitin-directed VCP is recruited to ruptured lysosomes modified with the K48-linked ubiquitin chain, in order to promote autophagosome formation (Fig. 1).[8] VCP mutants, such as R155Ht, have exhibited lysophagy processes that can cause inclusion body myositis and neurodegeneration.

The role of mitophagy in cancer development remains controversial. In hypoxia, the degradation of serine/threonine-protein kinase induced by mitogen-activated protein kinase 1 and 3 (MAPK1/3) decreases the mitophagy ability. The accumulation of damaged mitochondria leads to the activation of the NLRP3 inflammasome, which is critical for cancer metastasis.[11] However, in Smad family member 4-deficient cells, MAPK/extracellular signal-regulated kinase (MAPK/ERK) signaling drives the mitophagic flux, and confers cancer cell resistance to mitochondrial therapeutic agents.[12] For the critical role of VCP in organelle-specific autophagy, developing VCP inhibitors would help to overcome organelle-mediated drug resistance in clinical cancer therapy.

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Conflict of interest

One of the authors, Dr. Yang Wang, has been an editorial board member of the *Journal of Exploratory Research in Pharmacology* since February 2021. The authors have no other conflicts of interest.

Author contributions

JYF, JRZ and ZJH conceived and wrote the manuscript. YW designed and performed the writing-review, and undertook the editing and supervision.

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