TORC2-SGK-1 signaling integrates external signals to regulate autophagic turnover of mitochondria via mtROS

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ABSTRACT

Macroautophagy/autophagy is an evolutionarily conserved cellular degradation and recycling process that is tightly regulated by external stimuli, diet, and stress. Our recent findings suggest that in C. elegans, a nutrient sensing pathway mediated by MTORC2 (mechanistic target of rapamycin kinase complex 2) and its downstream effector kinase SGK-1 (serum- and glucocorticoid-inducible kinase homolog 1) suppresses autophagy, involving mitophagy. Induced autophagy/mitophagy in MTORC2-deficient animals slows down development and impairs reproduction independently of the SGK-1 effectors DAF-16/FOXO and SKN-1/NFE2L2/NRF2. In this punctum, we discuss how TORC2-SGK-1 signaling might regulate autophagic turnover and its impact on mitochondrial homeostasis via linking mitochondria-derived reactive oxygen species (mtROS) production to mitophagic turnover.

Changes in environmental conditions (stress) and aging may induce autophagy to maintain cellular homeostasis under unfavorable conditions. MTOR (mechanistic target of rapamycin kinase) and INS (insulin)-IGF (insulin like growth factor) are two of the prominent signaling pathways sensing shifts in nutrient availability, and function as critical autophagic regulators. Both pathways cross-talk with one another, however, many details of such crosstalks are still unknown. The transcription factor FOXO is the main effector of INS-IGF signaling, and its activity promotes autophagy induction. The kinase MTOR forms the enzymatic core of two distinct, but highly conserved protein complexes, MTORC1 and MTORC2. MTORC1 is a well-studied, potent repressor of predominantly other physiological responses.

Using reporters to monitor autophagy, we found that rict-1 and sgk-1 deficiency results in a strong increase of autophagy in different tissues, suggesting that MTORC2, like MTORC1, counteracts autophagy in C. elegans [1]. This observation was confirmed by knocking down additional components of MTORC2. Surprisingly, autophagy induction is independent of a prominent SGK-1 target, the FOXO transcription factor DAF-16. daf-16 mutants, although fully suppressing the autophagy induction resulting from loss of akt-1;akt-2 that act in parallel to sgk-1 in the INS-IGF pathway, do not have an impact on rict-1(-/-)-induced autophagy.

Because our previous experiments had suggested that mutant sgk-1 animals have moderately increased unfolded protein responses of the mitochondria (mtUPR) that might induce mitophagy, we next tested whether mitophagy was responsible for increasing autophagosomes in MTORC2-deficient animals. Fluorescent marker screens indeed suggested an increased sequestration of mitochondria that resulted in delivery to acidic autolysosomes.

Targeting defective mitochondria to the autophagosome requires the mitochondrial outer membrane interactors of LGG-1/LC3, DCT-1/BNIP3/NIX, PINK-1 and PDR-1/PRKN/Parkin. Mutants of each of these genes reduce puncta in rict-1- and sgk-1-deficient animals, implying that mutant MTORC2 indeed increases mitophagy. Both sgk-1 and rict-1 mutants display developmental retardation and low brood sizes. We found that these phenotypic aspects can partially be suppressed by inhibiting mitophagy.

Do sgk-1 and rict-1 animals suffer from perturbed mitochondrial homeostasis that, in addition to activating the mtUPR, induces mitophagy? Apparently this is the case, because these mutants exhibit altered oxygen consumption rates and a strong reduction in TMRE staining, a dye that labels...
active mitochondria. Therefore, increased mitochondrial depolarization, low membrane potential ($\Delta \Psi_m$) and an aberrantly increased mitophagy might contribute to the reproductive and developmental phenotype of MTORC2-deficient animals.

A low $\Delta \Psi_m$, conversely, should result in reduced ROS generation as a byproduct. However, MTORC2 mutants show greatly enhanced mitochondrial ROS levels that might diffuse to the cytoplasm to trigger autophagy. Indeed, sgk-1 and rict-1 deficiency induce cytosolic ROS. Scavanging by NAC reduces the number of autophagic puncta and also alleviates developmental defects in MTORC2 mutants. Thus, increased ROS levels induce mito/autophagy in MTORC2-deficient animals. Surprisingly, this induction does not depend on SKN-1/NFE2L2, the critical regulator of the phase II detoxification of cytosolic ROS that we and others have shown previously to be activated upon sgk-1 downregulation. Instead, it requires HIF-1 (hypoxia inducible factor), CEP-1/TP53/p53, and AMPK, all of which have been previously implicated in autophagy regulation. In summary, these data suggest that MTORC2 might steer a novel transcriptional program that does not rely on DAF-16/FOXO or SKN-1/NFE2L2.

What are the candidate phosphorylation targets of altered RICT-1 and SGK-1 activity? In unpublished experiments, we had identified a short list of candidates, among them VDAC-1, a mitochondrial outer membrane protein proposed to function in the mPTP complex. VDAC-1 was recently confirmed by the Soukas lab as a substrate of SGK-1, suggesting that SGK-1 negatively regulates VDAC-1 by promoting its turnover. According to this model, loss of MTORC2 activity might therefore result in an uncontrolled opening of the mitochondrial permeability transition pore. How this could activate mitophagy is still not known, but we suggest that under low MTORC2 signaling, mtROS might couple mitochondrial perturbations to autophagy induction.

There are a number of unsolved questions in the mysteries around sgk-1 and rict-1. Both our results and that of the Soukas lab suggest that MTORC2 deficiency decreases the mitochondrial membrane potential ($\Delta \Psi_m$), whereas other studies had suggested an increase in $\Delta \Psi_m$ in RICTOR mutants in human cancer cells and mouse keratinocytes. However, as indicated above, loss of rict-1 and sgk-1 may have adverse phenotypic consequences already in C. elegans, depending on alterations of external stimuli such as food source and temperature. Nevertheless, our experiments show that altered mitochondrial redox signaling plays a key role in autophagy regulation downstream of MTORC2, suggesting a mitohormetic function of ROS that differentially affects mitochondrial function and signaling during stress versus non-stress conditions.

Increasing autophagy by a number of measures are currently being proposed for treatment of many diseases, including cancer and neurodegeneration. MTORC2 inhibitors have already been discussed for the treatment of solid cancers, myelomas and lymphomas. In the light of malicious autophagy induction reported here, these should be considered with caution!

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