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Functions of ULK1 in autophagy and non-autophagy pathways and its implications in human physiology and disease

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Abstract: ULK1 (unc-51 like autophagy activating kinase 1), a mammalian serine/threonine kinase, is a key component of autophagy initiation complex and helps to induce all types of autophagy. Canonical autophagy is a process in which, through the interactions of a series of autophagy-related proteins, damaged organelles or misfolded proteins are engulfed by autophagosomes and then merged with lysosomes to be degraded. Thus, canonical autophagy is an important constituent part of the cellular "quality control." Besides, accumulating evidence indicates that ULK1 exerts autophagy-independent effects in a cell-specific manner. For example, ULK1 facilitates neurite elongation through the regulation of endoplasmic reticulum (ER)–Golgi trafficking in neurons, stimulates phosphopentose pathway to help NADPH (nicotinamide adenine dinucleotide phosphate hydrogen) production, and acts as a duplex regulator in type I IFN (type I interferon) induced innate immune response. Considering the importance and diversity of ULK1 in various biological processes, this review aims to present a comprehensive overview of autophagy and non-autophagy related functions of ULK1 in a variety of human physiological, pathological, and disease processes.

Introduction

ULK1 is a mammalian serine/threonine kinase comprising a kinase activation domain in the N terminus, a serine/proline-rich area in the central domain, and a conserved domain with PDZ-binding motif in the C terminus (Dorsey et al., 2009). In mammals, ULK1 ordinarily binds to FIP200 (focal adhesion kinase family interacting protein of 200kD), ATG13 (autophagy-related 13), and ATG101 (autophagy-related 101) to form a complex named the autophagy initiation complex or ULK1-ATG13-FIP200-ATG101 complex that initiates autophagy (Jung et al., 2009; Mercer et al., 2009).

The alternation of any component of the autophagy initiation complex can induce changes in the complex itself or its downstream biological responses. For instance, FIP200 deletion can spoil the structural stability and phosphorylation reaction of ULK1 (Hara et al., 2008); ATG13 binding to ULK1 can enhance the stability of ULK1, and can reinforce the phosphorylation of FIP200 by ULK1 (Hosokawa et al., 2009a; Jung et al., 2009); ATG101 can directly bind to HORMA (Hop1p, Rev7p and MAD2 proteins) domain of ATG13 and prevent it from lysosomal

degradation (Hosokawa et al., 2009b; Suzuki et al., 2015); etc. Meanwhile, the C terminus of ATG101 can act as a bridge connecting ULK1 with PI3KC3-C1 (class III phosphatidylinositol 3-kinase complex I), a downstream complex of ULK1 in the autophagy pathway (Kim et al., 2018). ATG101 can also recruit other downstream factors to autophagosomes and boost their formation and final maturation (Suzuki et al., 2015).

The fluctuation of certain components, such as glucose, amino acid, and oxygen, in the internal environment, can induce the activation or deactivation of cellular AMPK (adenosine monophosphate-activated protein kinase) and mTORC1 (mammalian target of rapamycin complex, 1). Both of these compounds can phosphorylate different phosphorylation sites of ULK1 and induce different consequences of autophagy, induction, or suppression (Ganley et al., 2009; Kim et al., 2011). The communication of AMPK and mTORC1 with ULK1, in autophagy, has been extensively studied to explain the effect of energy changes on autophagy and the redistribution of nutrients by autophagy. Besides, the structural analysis of activated ULK1 showed that the serine residue 180 of ULK1 is auto-phosphorylated following ULK1 activation, implying that auto-phosphorylation may contribute to ULK1 activation (Lazarus and Shokat, 2015). Overall, the phosphorylation or dephosphorylation of ULK1 induced by

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AMPK and mTORC1, as well as ULK1 auto-phosphorylation, can regulate autophagy.

ULK1 mediated autophagy works both physiologically and pathologically. It facilitates energy metabolism and reticular maturation (An et al., 2017; Kundu et al., 2008; Li et al., 2016; Ro et al., 2013), maintains tumor cell survival (Dower et al., 2018; Follo et al., 2018; Jiang et al., 2014; Jiang et al., 2011; Lu et al., 2018; Zhang et al., 2017), and participates in the stress reaction in various types of cells (Ci et al., 2014; Joshi et al., 2016; Lin et al., 2012; Mukhopadhyay et al., 2015; Nie et al., 2016). Moreover, for years, other roles of ULK1 in different facets besides autophagy have been under investigation. ULK1 can help in axonal extension (Desai et al., 1988; Hedgecock et al., 1985; McIntire et al., 1992; Ogura et al., 1994; Siddiqui, 1990; Tomoda et al., 1999) and regulate innate immune responses (Konno et al., 2013; Saleiro et al., 2018; Saleiro et al., 2015), which all bypass the canonical autophagy pathway. Considering the importance of ULK1, this work aimed to summarize the advanced knowledge about ULK1 functions in human physiological, pathological, and disease processes via autophagy and non-autophagy pathways.

The Mechanism of ULK1 Mediated Canonical Autophagy Pathway

AMPK-ULK1-PI3KC3-C1-mediated autophagy activation pathway

AMPK is a highly conserved energy checkpoint in eukaryotes. After exhaustion of cellular glucose and a drop in cellular ATP levels, AMPK is activated and regulates the activity of multiple metabolic enzymes to suppress anabolism and promote catabolism (Egan et al., 2011). In this process, AMPK phosphorylates the serine residues 317 and 777 of ULK1 to induce ULK1 kinase activity (Kim et al., 2011). ULK1 can concurrently occur auto-phosphorylation in this process (Lazarus and Shokat, 2015). After activation, ULK1 can also phosphorylate FIP200 and ATG13 in the autophagy initiation complex (Jung et al., 2009). The phosphorylation processes activate the autophagy initiation complex and initiate the canonical autophagy pathway.

Activated autophagy initiation complex will further phosphorylate the threonine residues 14 (Russell et al., 2013) and 30 (Park et al., 2018) of ATG6/Beclin-1 (autophagy-related protein-6) to activate PI3KC3-C1. PI3KC3-C1 is the second key complex in the autophagy pathway (Hurley and Young, 2017). ATG6/Beclin-1 is one of the four subunits of PI3KC3-C1, and the other three subunits are respectively VPS34/PIK3C3 (phosphatidylinositol 3-kinase catalytic subunit type 3), VPS15/PIK3R4 (phosphoinositide-3-kinase regulatory subunit 4), and ATG14 (autophagy-related 14) (Ma et al., 2017). Activated PI3K3-C1 further phosphorylates the three phosphorylation sites of phosphatidylinositol to produce numerous PI3P (phosphatidylinositol 3-phosphate) (Kim et al., 2013). PI3P is not only the fundamental constituent of the phagophore but also recruits other autophagy-related molecules to help autophagosomal nucleation and expansion (Kim et al., 2013). In the process of extension,

phagophores encompass deserted protein and organelles in the cytoplasm, and subsequently merge with lysosome forming autophagolysosomes; the deserted protein and organelles will then be degraded by lysosomal enzymes (Mizushima and Komatsu, 2011). ULK1 can recruit STX17 (syntaxin-17) to the fusion location and enhance the affinity of STX17 to SNAP29 (synaptosomal-associated protein-29) to encourage the fusion of autophagosome and lysosome (Wang et al., 2018). However, both AMPK and ULK1 are not merely positive enhancers of autophagy. It was found that ULK1 could phosphorylate all three subunits of AMPK, AMPKα, AMPKβ, and AMPKγ in a negative feedback loop to inhibit AMPK activity as a manner to control the duration and amplitude of autophagy (Nwadike et al., 2018). Besides, AMPK can also phosphorylate the serine residue 555 of ULK1 to inhibit autophagosome formation (Laker et al., 2017).

Co-factor is indispensable for the proper functioning of PI3KC3-C1. For example, Ambra1 (the activating molecule in Beclin 1 regulated autophagy-1) can bind to both ULK1 and PI3KC3-C1 to ensure the interaction between the autophagy initiation complex and PI3KC3-C1. It can also sustain the structural stability and kinase activity of ULK1 (Nazio et al., 2013). Besides, Ambra1 can be phosphorylated by ULK1, which further induces the trans-location of Ambra1-PI3KC3-C1 combination from the cytoskeleton to the endoplasmic reticulum (ER) (Di Bartolomeo et al., 2010).

After being phosphorylated by AMPK, a portion of ULK1 in the cytoplasm can move to the mitochondria and boost the mitophagy pathway (Tian et al., 2015). Mitophagy is a specific form of autophagy, in which the cell degrades aging or impaired mitochondria in an autophagic manner. In the mitochondria, Hsp90 (hot shock protein-90), in combination with its co-chaperone Cdc37 (cell division cycle control protein-37), is able to bind with ULK1 and sustain its stability and kinase activity to guarantee mitophagy (Joo et al., 2011). On the outer membrane of the mitochondria, ULK1 can phosphorylate the serine residue 17 of mitochondria outer membrane protein FUNDC1 (FUN14 domain containing 1), which accelerates the interaction of FUNDC1 with LC3 (microtubule-associated protein 1 light chain 3 alpha) and promotes mitophagy (Wu et al., 2014). Besides, it was found that PRPF8 (pre-mRNA processing factor 8) can protect the proper function of mitophagy by maintaining the correct cut or splicing of ULK1 mRNA during DNA transcription (Xu et al., 2018).

MTORC1-ULK1 mediated autophagy suppression pathway

MTORC1 is also an important energy metabolism hub in eukaryotes. In contrast to AMPK, the main role of mTORC1 is to foster anabolism and promote cell growth and proliferation (Ganley et al., 2009). Under normal metabolic status, the raptor subunit of mTORC1 combines with ULK1, and the mTOR subunit of mTORC1 phosphorylates the threonine residue 757. This phosphorylation inactivates ULK1 and blocks the association of ULK1 with AMPK (Hara et al., 2002). However, when there is a deficiency of cellular amino acids, especially glutamine, leucine, and arginine, mTORC1 will dissociate from ULK1, and ULK1, reunite with AMPK and restore its kinase activity to initiate autophagy pathway (Yu et al., 2010).

Besides, ULK1 can also exert a negative impact on mTORC1. It was found that ULK1 could phosphorylate serine residues 696, 792, 855, 859, 863, and 877, and threonine residues 706 of raptor subunit to inhibit mTORC1 activity (Dunlop et al., 2011). In addition, there is an inhibition of cell proliferation after ULK1 induced raptor phosphorylation and mTORC1 pathway inhibition (Jung et al., 2011).

Ubiquitination is the major degradation method for ULK1
Ubiquitination is one of the most common protein degradation strategies, in which ubiquitin ligases add ubiquitin to a targeted protein and deliver it to the proteasome for degradation. Ubiquitination is reversible, i.e., ubiquitinated protein can be deubiquitinated through deubiquitinases to avoid proteasomal degradation (Popovic et al., 2014).

Previous researches on ULK1 degradation have all pointed to ubiquitination. At first, it was found that deubiquitinase inhibitor WP1130 could promote ULK1 ubiquitination and transference to aggresomes (Driessen et al., 2015). Moreover, there are numerous reports showing that ULK1 degradation is dependent on ubiquitination. Four E3 ubiquitin ligases have been reported to ubiquitinate and degrade ULK1 and lead to termination of autophagy. E3 ubiquitin ligase Cul3-KLHL20 (cullin 3-kelch like family member 20) can directly ubiquitinate ULK1 with its kelch repeat domain and degrade ULK1 in the ubiquitin–proteasome pathway (Liu et al., 2016). E3 ubiquitin ligase NEDD4L (neural precursor cell expressed, developmentally down-regulated 4-like) can ubiquitinate lysine residues at 925 and 933 of ULK1 (Nazio et al., 2016). E3 ubiquitin ligase TRAF6 (TNF receptor-associated factor 6) can ubiquitinate lysine residue 63 of ULK1 in an Ambra1 (a co-factor of PI3KC3-C1) dependent manner, while mTORC1 subunit mTOR can stop this process by phosphorylating the serine residue 12 of Ambra1 (Nazio et al., 2013). During mitophagy, the mitochondria located E3 ubiquitin ligase MUL1 (mitochondrial E3 ubiquitin-protein ligase 1) can directly ubiquitinate and degrade ULK1 that migrates from other locations in the cytoplasm to control mitophagy in a moderate status (Li et al., 2015a).

In contrast, there are three deubiquitinase, USP1 (ubiquitin specific peptidase 1) (Raimondi et al., 2019), USP20 (ubiquitin specific peptidase 20) (Kim et al., 2018b), and USP24 (Thayer et al., 2019), found to be able to combine with ULK1 and prevent it from ubiquitination and proteasomal degradation.

Despite the fact that merely ubiquitination degradation pathways have been confirmed to clear up ULK1 molecule in the cytosol, the possibility of discovering new degradation methods, especially those in lysosomal pathways or caspase pathways, should not be ruled out.

ULK1 Mediated Physiological and Pathological Processes in Autophagy Pathway

ULK1 mediated autophagy protects neurons from damages
ULK1 mediated canonical autophagy pathway in the central nervous system helps protect neurons from apoptosis (Lee and Tournier, 2011). As the main role of ULK1-mediated autophagy in cells is to clear deserted or pathological protein or organelles, this function could help neurons to resist damages in some morbid environments. In PD (Parkinson's disease) murine models, the specific ULK1 activator 33i (BL-918) induces autophagy to help remove Lewy bodies in dopaminergic neurons and maintain the number of living neurons (Ouyang et al., 2018). Besides, ULK1 can suppress tyrosine 389 phosphorylation of RPS6KB1 (ribosomal protein S6 kinase polypeptide 1) to resist cellular apoptosis and sustain cell viability (Li et al., 2015b). Furthermore, ULK1-mediated autophagy can protect prefrontal cortex from chronic intermittent ethanol-induced injuries in alcohol intoxication models (Sumitomo et al., 2017), protect auditory cortex from D-gal induced apoptosis in aging models (Yuan et al., 2018), and protect PC-12 cells from hypoxia-induced apoptosis (Wang et al., 2018c). The deficiency of ULK1-mediated autophagy participates in the pathology of several diseases of the central nervous system. For example, GGGCCC mutation in C9orf72 gene can cause a specific type of ALS (amyotrophic lateral sclerosis), and FD (frontotemporal dementia) C9ALS/FD, in which normal C9orf72 in neuron decreases, resulting in decreased activation and binding to Rab1a, finally disabling ULK1 complex translocation to phagophore and autophagy (Webster et al., 2016). Autophagy deficit is also one of the causes of PD (Lynch-Day et al., 2012).

ULK1 mediated autophagy is pro-survival for cancer cells
The role of ULK1 mediated autophagy during tumorigenesis is thought to be always pro-survival, irrespective of pre-cancer cells or tumor cells. At the beginning of tumorigenesis, when a normal cell is in hypoxic condition, ER stress and other oncologic factors elevating autophagy accelerate the removal of poisonous substances, misfolded or unfolded proteins, and damaged organelles to prevent the transformation of normal cells to tumor cells. When a neoplasm is already formed, the high replication, multi-mutation, and high metabolism state of the tumor greatly increase cellular protein burden. However, in this period, strengthened ULK1-mediated autophagy could still function as it is in pre-cancer cells to guarantee tumor cell survival (White, 2015; Zhong et al., 2016).

For example, ULK1 inhibitor SBI-02006965 markedly suppresses cellular proliferation and viability and induces cellular apoptosis of neuroblastoma (Dower et al., 2018) and renal clear cell tumor (Lu et al., 2018), and ULK1 knockdown in tumor xenograft improves the survival of nude mouse models. ULK1 inhibitor MRT68921 greatly increases chemotherapy sensitivity of an in vitro 3D model of mesothelioma (Follo et al., 2018). Besides, ULK1 protein expression is negatively correlated with patient's prognosis in prostate cancer (Zhang et al., 2017a), esophageal squamous cell carcinoma (Jiang et al., 2014; Jiang et al., 2011), hepatic cellular cancer (Xu et al., 2013), and renal clear cell tumor (Nishikawa et al., 2015). The above-mentioned results demonstrate that in established cancer, ULK1-mediated autophagy is conducive to maintain tumor cell survival and to withstand medical therapies, and the suppression of ULK1-mediated autophagy specifically in tumor cells could be a promising target for anti-tumor molecular therapy.

However, in two specific types of cancers, the relationship between ULK1 mediated autophagy and

neoplasm is confusing. In breast cancer, the expression of ULK1 is actually down-regulated and ULK1 expression is negatively correlated with tumor size, pathological stage, and lymph node metastasis, while positively correlated with patient survival (Tang *et al.*, 2012). Besides, ULK1 activator LYN-1604 is sufficient to induce the death of breast cancer cells (Ouyang *et al.*, 2017; Zhang *et al.*, 2017b). Moreover, the protein expression of ULK1 is also positively correlated with the survival of gastric cancer patients (Chen *et al.*, 2017). It appears that in these cancers, ULK1 is suppressed, which is surprisingly in favor of tumor development. It is found that during DNA damage, tumor suppressor p53 can directly and transcriptionally activate ULK1 and induce prolonged autophagy (Gao *et al.*, 2011). Oncoprotein PPMID (protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1D) can phosphorylate serine 15 of p53 to induce p53 degradation and de-phosphorylate serine 673 of ULK1, and altogether suppress ULK1 mediated autophagy (Torii *et al.*, 2016). Therefore, it is possible that in specific types of cancers, some activated oncoproteins suppress ULK1 and autophagy-related molecules and can induce other substitute pathways to maintain tumor survival and development.

ULK1 mediated autophagy facilitates lipid metabolism and hematologic maturation

ULK1 mediated autophagy takes part in lipid metabolism in a process known as lipophagy, which helps eliminate lipid substances. Cardiac specific ULK1 knockout murine models exhibited an accumulation of lipoprotein lipase in cardiomyocytes, which might strengthen the risk of cardiovascular diseases, while the enhancement of autophagy capacity in cardiomyocytes in obese murine models can effectively decrease the accumulation of lipoprotein lipase and triglyceride (An *et al.*, 2017). Specific ULK1 knockdown in adipocyte could lower basal lipolysis and decrease the uptake and synthesis of fatty acid in adipocyte (Ro *et al.*, 2013). Hence the maintenance of ULK1 is important for lipid metabolism.

One of the main characteristics of reticulocyte maturation is the removal of mitochondria, ribosomes, and other organelles. In addition, ULK1-mediated mitophagy might take part in the removal of mitochondria during development from reticulocyte to mature red cells. It is found that the expression level of cellular ULK1 is elevated in pace with the increased proportion of orthochromatic erythroblasts to erythroblasts. Further, ULK1 knockdown will cause delayed or failed clearance of mitochondria, RNA retention, and disappeared CD71 expression in reticulocytes (Kundu *et al.*, 2008).

ULK1 Mediated Physiological and Pathological Processes in Non-autophagy Pathway

ULK1 contributes to axonal extension

The earliest known function of ULK1 was actually found in the neurons of nematode *Caenorhabditis elegans*, where it is called unc-51 and works to promote axonal development in an autophagy-independent manner. It was in *Caenorhabditis elegans* that natural mutation of unc-51 was firstly found, and mutant unc-51 induced paralysis

(Brenner, 1974). In an artificial unc-51 deletion nematode, the axons of three types of sensory neurons, amphid neurons, phasmid neurons, and posterior deirid neurons all showed premature termination and abnormal extension of varying degrees (Desai *et al.*, 1988; Hedgecock *et al.*, 1985; McIntire *et al.*, 1992; Ogura *et al.*, 1994; Siddiqui, 1990; Tomoda *et al.*, 1999). Various studies have been conducted to investigate how unc-51 affects axonal development, and they indicate that unc-51 might be involved in vesicular trafficking of specific molecules. In motor neurons of nematode, unc-51 with its binding partner unc-14 (Ogura *et al.*, 1997) controls the formation, selection, and transportation of unc-5 related small vesicles from soma to axon (Ogura and Goshima, 2006). Unc-51 can bind to KHC (kinesin heavy chain) receptor protein unc-76 and phosphorylate its serine 143. The phosphorylated unc-76 is then activated and binds to synaptic vesicle protein synaptotagmin-1, facilitating the connection between the synaptic vesicle and motor protein (Toda *et al.*, 2008).

The study of ULK1 in the nervous system was naturally extended to the mammalian mouse model. In murine neurons, ULK1 is located at the vesicular structures in the axonal shafts and growth cones of extending axons (Tomoda *et al.*, 1999). Genetic mutation of ULK1 causes aberrant distribution and over-fasciculation of corticothalamic axons and thalamocortical axons when crossing the pallial-subpallial boundary in the embryonic stage and causes overfasciculation and hypoplasticity of corpus callosum fibers after birth (Wang *et al.*, 2018a). ULK1 is one of the molecules that participate in the regulation of early differentiation of cerebellar granule cells, the kinase domain inactivation of which causes the suppression of axonal growth (Tomoda *et al.*, 1999). In the PHEV (porcine hemagglutinating encephalomyelitis virus)-induced central nervous system degeneration model, the expression of ULK1 was suppressed. However, restoration of ULK1 expression could strengthen neurite outgrowth and regeneration (Wang *et al.*, 2018c). It appears that ULK1 controls axonal extension, guidance, and maturation during early central nervous system development and helps maintain axonal integrity through adulthood. Many studies have been conducted to determine the mechanism underlying the influence of ULK1 on mammalian axonal development. In the axon of murine cerebellar granule cells, ULK1 can bind to SynGAP (synaptic Ras GTPase activating protein) and suppress the SynGAP-Ras-Rab5 pathway to prompt the merger and retrograde transportation of Rab5-mediated endosomal membranes (Tomoda *et al.*, 2004). ULK1 can bind to SEC16A and phosphorylate it at serine 846 at ER exit sites (ERESs), which promotes SEC16A activation and SEC16A-induced COPII assembly to facilitate further ER-Golgi trafficking of specific molecules such as serotonin (Joo *et al.*, 2016). In murine spinal sensory neurons, ULK1 induces clathrin-independent endocytosis of NGF (nerve growth factor). The engulfed NGF fosters K63-polyubiquitination of ULK1 in a TRAF-dependent manner, resulting in the formation of a complex consisting of polyubiquitinated ULK1, scaffold protein p62, and NTRK1 (neurotrophic tyrosine receptor kinase 1), which further attenuates the Akt pathway to suppress excess filopodia

elongation and neurite branching (Zhou et al., 2007). In brief, ULK1 has a conserved function in maintaining vesicular trafficking in axons, which is necessary for the normal morphology and functioning of axons.

In brief, the function of ULK1 in the central nervous system can be divided into two parts. First, ULK1 helps in anterograde or retrograde trafficking of specific types of vesicles to sustain axonal homeostasis both at the developmental and maturation stages. Second, ULK1 also initiates canonical autophagy, which helps in removing toxic proteins or deserted organelles to protect neurons from unfavorable environmental conditions.

ULK1 induces the phosphopentose pathway to increase NADPH production

As mentioned above, glucose deficiency can induce ULK1-mediated autophagy (Kim et al., 2011). ULK1 can also facilitate the occurrence of the phosphopentose pathway in an autophagy-independent manner. Activated ULK1 can bind to and phosphorylate several key enzymes of glycolysis including hexokinase, phosphofructokinase-1, fructose-1,6-bisphosphatase, and enolase-1, which enhance the occurrence of the phosphopentose pathway and increase NADPH/DECR1 (2,4-dienoyl-CoA reductase 1) production; NADPH can further provide a reducing agent for cellular reduction reactions (Li et al., 2016).

Therefore, ULK1 is not only influenced by fluctuation in the levels of glucose and other nutrients in the internal environment but can also affect lipid metabolism and the phosphopentose pathway via autophagy-independent and -dependent manners, respectively. Therefore, ULK1 can aid cell survival by regulating cellular energy recycling.

ULK1 is a duplex regulator in type I IFN-mediated innate immune response

The human innate immune system is responsible for eliminating invading microbial pathogens, and this is largely dependent on type I IFN. This function of type I IFN has been exploited in medical therapies to control viral infection, immune disorder, and malignant diseases (Gonzalez-Navajas et al., 2012; Platanias, 2005). ULK1 functions as a positive intermediary, which reinforces type I IFN-induced innate immunization in an autophagy-independent manner.

During type I IFN-mediated innate immune response, type I IFN can, in an Akt-dependent manner, induce the phosphorylation of serine residue 757 of ULK1, which is also the phosphorylation site of mTORC1. Different from mTORC1-induced ULK1 inactivation, ULK1 is activated by type I IFN. In p38 MAPK (p38 mitogen-activated protein kinase) dependent manner, type I IFN promotes the transcriptional activation of multiple ISGs (IFN-stimulated genes) including IRGM2 (immunity-related GTPase family M member 2), GCH1 (GTP cyclohydrolase 1), IFIT3 (interferon-induced protein with tetratricopeptide repeats 3), OASL2 (2'-5' oligoadenylatesynthetase-like 2), IRF7 (interferon regulatory factor 7), IRF9 (interferon regulatory factor 9), and IFIT2 (interferon-induced protein with tetratricopeptide repeats 2) to enable the innate immune response (Saleiro et al., 2015). However, it is still unknown why phosphorylation by type I IFN and mTORC1 on the

same site would induce different ULK1 activation statuses. Besides, ULK1-mediated MAP3K11 (mitogen-activated protein kinase kinase kinase 11) and MAPK7 (mitogen-activated protein kinase 7) activation are also indispensable for the anti-viral ability of type I IFN (Saleiro et al., 2018).

In addition, ULK1 causes the regulation of STING (stimulator of interferon gene), one of the upstream molecules of type I IFN. STING is an ER located sensor that can detect aberrant cytosolic nucleic acids and shift to the nucleus to directly transcriptionally activate multiple type I IFN related molecules including type I IFN, IRF3 (interferon regulatory factor 3), and NF-κB (nuclear factor kappa B) for activation of type I IFN-related innate immune response (Burdette et al., 2011; Ishikawa et al., 2009; Woodward et al., 2010). However, during a sustained DNA injury, autophagy is found to be activated, and the activated ULK1 is able to phosphorylate threonine residue 366 of STING and inhibit STING activity, leading to lowered expression of type I IFN, IRF3 and type I IFN-related innate immune response (Konno et al., 2013).

Therefore, ULK1 intermediately helps type I IFN-mediated innate immune response by transcriptionally activating multiple ISGs. However, when an injury is sustained, ULK1 is able to directly target STING, upstream of type I IFN, to restrain the innate immune response and protect cells from excessive immune attacks.

ULK1-induced mitochondrial oxidative stress and ER stress

During mitophagy, after ULK1 enters the mitochondria, ULK1 can inhibit the activity of MnSOD (manganese superoxide dismutase) and encourage the production of ROS (reactive oxygen species), which might further promote apoptosis (Mukhopadhyay et al., 2015). In addition, mitochondrial ROS accumulation can further decrease cellular p70S6K kinase phosphorylation, which inhibits the phosphorylation of serine residue 392 of p53 protein, decreases activated p53, and limits ULK1 transcription, leading to an attenuated autophagy reaction (Ci et al., 2014). Thus, oxidative stress appears to form a negative feedback loop with ULK1, and when the level of mitochondrial oxidative stress gets higher, the cell tends to undergo apoptosis rather than autophagy.

Besides, oxidative stress can contribute to ULK1 transportation to the nucleus. In the nucleus, ULK1 is able to stabilize the DNA damage repair protein PARP1 (poly (ADP-ribose) polymerase 1) and activate the post-transcription modification ability of PARP1 (Joshi et al., 2016), which accelerates cellular ATP consumption and apoptosis (Ha and Snyder, 1999). Notably, the role of ULK1 in the nucleus is independent of its roles in the autophagy pathway in the cytoplasm.

A variety of changes in the internal or external environment including hypoxia, hypoglycemia, and acidosis can lead to an increase in the number of unfolded or misfolded proteins in the ER, which further overloads the protein folding capacity of ER and finally causes ER stress (Rashid et al., 2015). Further, ULK1-mediated autophagy appears to protect cells from damages caused by ER stress. ER stress can decrease the phosphorylation level of serine residue 9 of GSK3β (glycogen synthase kinase 3 beta) and strengthen the phosphorylation of serine residue 86 of

TIP60 (Tat interactive protein 60 kD). The phosphorylation of serine residue 86 of TIP60 can promote the transacetylase activity of TIP60 and induce ULK1 acetylation and activation. The activated ULK1, and subsequent autophagy, leads to the degradation of unfolded or misfolded proteins, which in turn reduces ER stress and its negative effects on cell survival (Lin *et al.,* 2012; Nie *et al.,* 2016).

Therefore, both mitochondrial oxidative stress and ER stress are caused by disordered internal or external environments. According to the discrepant stress intensities, ULK1 plays different roles. When internal or external stress is mild or moderate, ULK1-mediated autophagy is enhanced to eliminate damaged organelles and misfolded proteins for the sustenance of cell survival. However, when there is increasing stress and the maximum self-digestion capacity of a cell has been reached, ULK1 can also work as an intermediate hub to transport signals for the programmed cell death process to avoid unnecessary energy wastage.

Conclusion

ULK1-mediated autophagy exists extensively in all kinds of cells in different organisms. The canonical pathway of autophagy regulated by AMPK and mTORC1 according to various nutritional environments helps in the digestion of deserted organelles or misfolded proteins, thereby maintaining cellular homeostasis. The ubiquitin-proteasomal pathway is a major ULK1 degradation pathway. ULK1-mediated autophagy helps in the elimination of pathological bodies in neurons, removal of excess lipid substances, maturation of red cells, and stabilization of oxidative or ER stress to ensure cellular survival, which is favorable for the organism in most circumstances. However, when these functions occur in tumor cells, ULK1-mediated autophagy would be an obstruction for medical strategies aiming to attack cancer cells. Therefore, tumor specific ULK1 knockdown might assist in cancer molecular therapy. Besides, ULK1 also functions independently of autophagy. It facilitates neurite elongation through regulation of ER-Golgi trafficking in neurons, stimulates the phosphopentose pathway to aid in NAPDH production, and works as a duplex regulator in the type I IFN-induced innate immune response. In fact, many of the organ- or tissue-specific functions of ULK1, especially its irreplaceable role in neurite regeneration, could aid in the development of therapeutic strategies for some clinical problems. In summary, ULK1 mediates multiple physiological or pathological processes in both an autophagic and non-autophagic manner and is, therefore, a promising target for molecular therapies in specific diseases.

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