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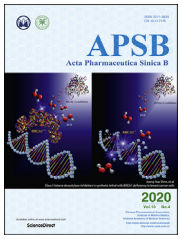

REVIEW

Targeting autophagy-related protein kinases for potential therapeutic purpose

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Abstract Autophagy, defined as a scavenging process of protein aggregates and damaged organelles mediated by lysosomes, plays a significant role in the quality control of macromolecules and organelles. Since protein kinases are integral to the autophagy process, it is critically important to understand the role of kinases in autophagic regulation. At present, intervention of autophagic processes by small-molecule modulators targeting specific kinases has becoming a reasonable and prevalent strategy for treating several varieties of human disease, especially cancer. In this review, we describe the role of some autophagy-related kinase targets and kinase-mediated phosphorylation mechanisms in autophagy

Abbreviations: 4E-BP1, eukaryotic translation initiation factor 4E-binding protein; AKT1, AKT serine/threonine kinase 1; AMBRA1, autophagy/beclin-1 regulator 1; AMPK, AMP-activated protein kinase; ARF, auxin response factor gene; ATG, autophagy-related protein; CaMKK2, calcium/calmodulin-dependent protein kinase kinase 2; DAPK, death associated protein kinase; FIP200, FAK family kinase-interacting protein of 200 kDa; GAP, GTPase-activating protein; GO, gene ontology; GSK3α, glycogen synthase kinase 3 alpha; HMGB1, high mobility group protein B1; JNK1, C-Jun N-terminal kinase; LC3, microtubule-associated protein 1 light chain 3; LKB1, serine/threonine-protein kinase stk11; LPS, lipopolysaccharide; LRRK2, leucine rich repeat kinase 2; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; PD, Parkinson's disease; PI, phosphatidylinositol; PI3 kinase, phosphoinositide 3-kinase; PI3P, phosphatidylinositol triphosphate; PIM2, proviral insertion in murine lymphomas 2; PINK1, PTEN-induced putative kinase 1; PIP2, phosphatidylinositol-4,5-bisphosphate; PKACα, a protein kinase cAMP-activated catalytic subunit alpha; PKCα, protein kinase C alpha type; PKD1, polycystin-1; PPIs, protein−protein interactions; PROTAC, proteolysis targeting chimeras; PTMs, post-translational modifications; Rheb, the RAS homolog enriched in brain; TAK1, transforming growth factor activated kinase-1; TFEB, transcription factor EB; TNBC, triple-negative breast cancer; TSC1/2, tuberous sclerosis complex proteins 1/2; ULK complex, ULK1−mATG13−FIP200−ATG101 complex; ULK1, unc-51-like kinase 1; UVRAG, ultraviolet resistance-associated gene.

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Human disease therapy

regulation. We also summarize the small-molecule kinase inhibitors/activators of these targets, highlighting the opportunities of these new therapeutic agents.

1. Introduction

Autophagy, first proposed in 1963 at the International Conference of Lysosomes by Belgian scientist Christian de Duve, refers to a highly conserved cellular self-digestion process by which cellular components are targeted for degradation *via* lysosomes. Autophagy in mammalian cells can be categorized into three main ways: macroautophagy, microautophagy, and chaperone-mediated autophagy[1−3]. Of these, macroautophagy (henceforth, autophagy) is the most intensively studied. In general, autophagy plays a Janus role and is implicated in certain human diseases[4,5]. For one thing, moderate autophagy is regarded as a cytoprotective mechanism. It governs the degradation of denatured proteins and nucleic acids in damaged, denatured, aging cells, organelles and biomacromolecules, which provide raw materials for cell regeneration and repair[6,7]. Also, autophagy can resist the invasion of pathogens and protect cells from detrimental cellular components. For another, excessive autophagy can contribute to metabolic stress, cell death, etc.

Accumulating research has indicated that protein kinases are integral to autophagy. Both autophagy initiation and autophagy signaling pathways utilize kinase mechanisms. An example of the latter is mammalian target of rapamycin (mTOR). Furthermore, the activity of these initiation complexes and signaling pathways is also highly dependent on post-translational modifications (PTMs)[8−10] including phosphorylation, ubiquitination, acetylation, glycosylation and lipidation. The PTMs can occur at multiple stages of autophagosome formation, leading to the induction, regulation and fine-tuning of autophagic responses. In particular, kinase-catalyzed phosphorylation reactions are by far the most thoroughly investigated components of autophagic PTMs[11]. Phosphorylation plays a role in regulating catalytic activity and protein−protein interactions (PPIs), and almost every signal transduction process (autophagy and beyond) is linked with a phosphate transport cascade. Thus, a specific physiological response can be induced by changing the activity of kinases, demonstrating their essential nature for human physiology. Typically, unc-51-like kinase 1 (ULK1, mammalian homologue of the yeast Atg1 kinase) has been identified as a significant autophagic initiator. ULK1 is the sole serine/threonine protein kinase in all known 38 autophagy-related proteins (ATGs). As an indispensable constituent of autophagy vesicles, ULK1 constitutes ULK1 complex with ATG13, FAK family kinase-interacting protein of 200 kDa (FIP200) and ATG101 to induce autophagy[12,13]. In the presence of amino acids, mammalian target of rapamycin complex 1 (mTORC1) is activated to inhibit autophagy by phosphorylating ULK1 and ATG13. However, during nutrient deficiency, mTORC1 on the lysosomal surface is inhibited thereby allowing ULK1 and ATG13 to be rapidly dephosphorylated, thus leading to the activation of ULK1 kinase and induction of autophagy[14].

Another case in point is phosphoinositide 3-kinase (PI3 kinase, the ortholog of yeast Vps34). Phosphorylation of phosphatidylinositol (PI) by PI3K produces phosphatidylinositol triphosphate (PI3P), a key membrane marker for both intracellular trafficking and autophagosome formation[15]. PI3K is activated by binding to serine/threonine-protein kinase Vps15 and further binding to beclin-1 to form the PI3K−Vps15−beclin1 complex. Within this complex, beclin-1 is phosphorylated by ULK1, which then acts as a scaffold of PI3K complex, promoting localization of autophagy protein to autophagy vesicles[16]. As such, PI3K kinase interacts with various regulatory proteins to form multiple complexes which will selectively participate in different stages of autophagy. For example, a complex of PI3K kinase and ATG14 is involved in the formation of autophagy vesicles[17]. When combined with ultraviolet resistance-associated gene protein (UVRAG), PI3K participated in the maturation and transportation of autophagic vesicles[18]. These findings indicate that decrypting the regulatory role of kinases in autophagy can facilitate a deeper understanding of these important mechanisms.

In this review, 49 autophagy-related kinases were mined by gene ontology (GO) analysis. These kinases are involved in autophagy regulation, mainly in autophagy initiation and the formation of autolysosome. Furthermore, we have interpreted in detail the role of some kinases in autophagy, and summarized related small-molecule kinase inhibitors/activators for autophagy induction and inhibition.

2. Identification of autophagy-related kinases

To identify kinases that are associated with autophagy, the keyword "autophagy" was used to perform a search for related GO terms on the Gene Ontology Consortium[19] website (http://www.geneontology.org). With the designated species as Homo sapiens, 499 resultant protein targets among 57 autophagy-related GO terms were obtained and then normalized, followed by a comparison between the normalized proteins and all 518 kinase proteins[20]. These results identified a total of 49 proteins as autophagy-related kinases (Table 1). Some of these kinases (*e.g.*, mTOR) are well studied, but little is known about many of the others. Their potential for autophagy regulation remains to be evaluated.

Table 1 Autophagy-related kinases in different kinase families.

Family	Autophagy-related kinase
AGC	AKT1, ROCK1, PKACα, PKCα, PKCβ
CAMK	AMPKα1, AMPKα2, LKB1, MARK2, DAPK1, DAPK2, DAPK3, PIM2, PKD1
CK1	VRK1
CMGC	CDC2, CDK5, CK2α2, GSK3A, ERK1, ERK7, JNK1
TK	ABL1, ABL2, FAK, MET, SRC, KDR
TKL	LRRK2, TAK1, RIPK2
Other	CaMKK2, TBK1, IRE1, NEK6, NEK9, PINK1, NRBP2, GCN2, PLK1, PLK2, PLK3, TLK2, ULK1, ULK2, ULK3, PIK3R4, FRAP, ATM

3. Autophagy-related protein kinases in autophagy regulation

Autophagic processes (Fig. 1), can be divided into five stages: autophagy initiation, membrane nucleation and phagophore formation, phagophore expansion, fusion with the lysosome to form autolysosome, and degradation of contents of the package[21]. These processes correspondingly are regulated by multiple ATGs and kinases. However, kinase participation in autophagy mainly occurs during autophagy initiation and in the first stage of autolysosome formation. Therefore, we describe below the role of autophagy-related kinases in autophagic mechanisms from upstream to downstream of autophagy signaling transduction (from PI3K/AKT to ULK1). In addition, we separate the kinases that regulate beclin-1 and these aforementioned targets. The regulatory phosphorylation mechanisms in autophagy are underlined in Table 2[15,16,22–49].

3.1. PI3K/AKT

PI3K kinases, the most upstream molecules, act as triggers of autophagy signaling cascades, which are activated by tyrosine kinase receptors (RTKs), G protein-coupled receptors (GPCR) or Ras-like protein (RAS)[50]. Importantly, activation of PI3K leads to the generation of phosphatidylinositol-3,4,5-trisphosphate (PIP3), an anchor for phosphoinositide-dependent kinases 1 (PDK1), converted from phosphatidylinositol-4,5-bisphosphate (PIP2). Subsequently, PDKs phosphorylate AKT at Thr308[22]. The activated AKT signaling pathway is thus directly phosphorylated and thereby inhibits tuberous sclerosis complex proteins 1/2 (TSC1/2), a GTPase-activating protein (GAP) for the Ras homolog enriched in brain (Rheb) GTPase. The AKT-dependent phosphorylation causes the dissociation of TSC1/2 from lysosome and resultant activation of Rheb[23]. Since GTP-bound Rheb is a potent mTORC1 activator, suppression of TSC1/2 by AKT-dependent phosphorylation results in mTORC1 activation.

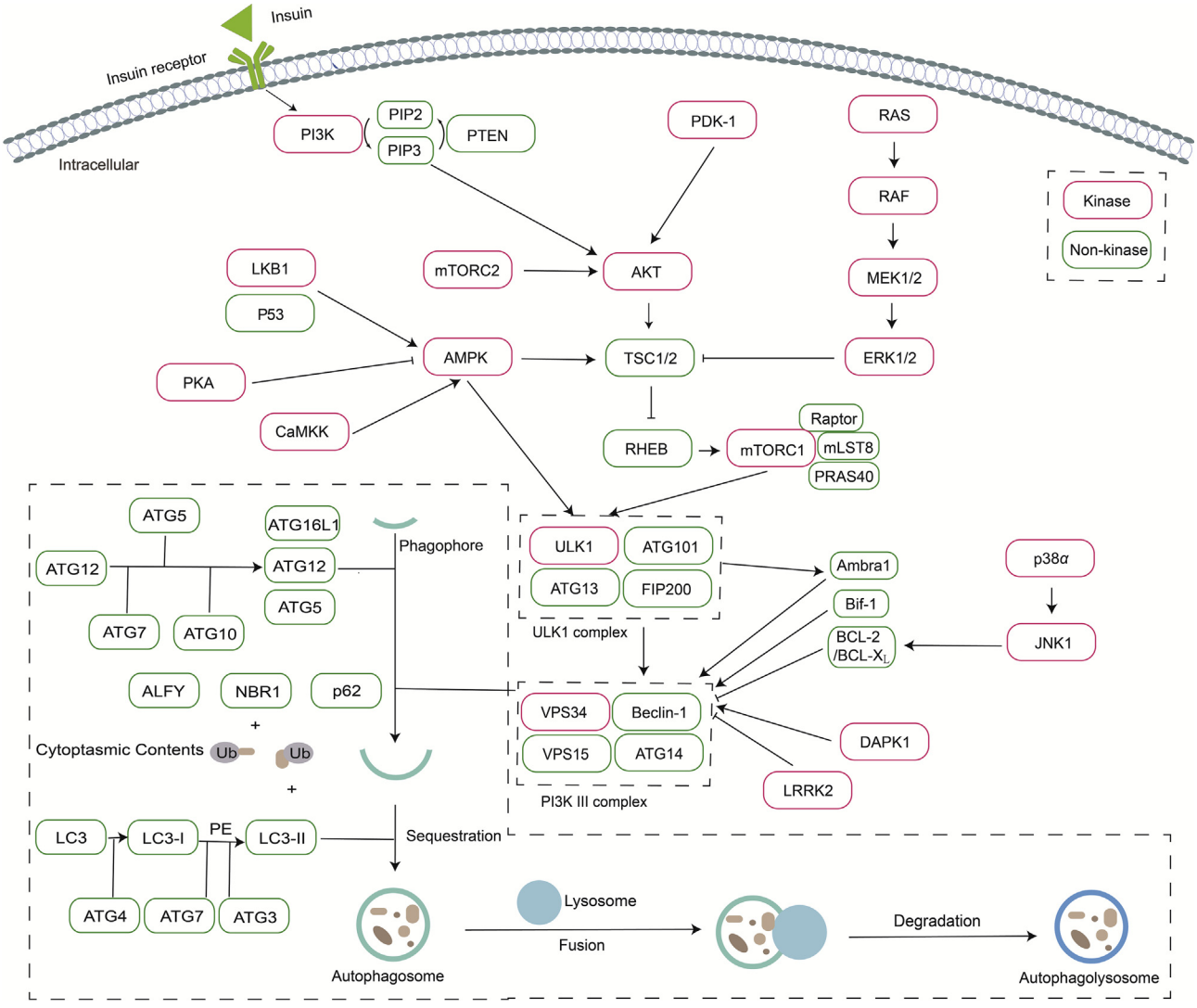

Figure 1 The relationship between autophagy processes and autophagy-related kinases. Autophagic processes can be divided into five stages: autophagy initiation, membrane nucleation and phagophore formation, phagophore expansion, fusion with the lysosome to form autolysosome, and degradation of contents of the package. Regulation of kinases in autophagy mainly occurs in autophagy initiation. During this step, several kinases are implicated in autophagy regulation, such as PI3K, AKT, AMPK, mTOR, and ULK1.

Table 2 Phosphorylation regulation of autophagy-related kinases.

Kinase	Substrate	Site	Function	Ref.
PI3K	PIP2	–	Forms second messenger PIP3	15
PDK1	AKT	Thr308	Activates AKT signaling	22
AKT	TSC2	Ser939/Thr1462	Dissociates TSC1/2 from lysosome and activates Rheb	23
AMPK	TSC2	Thr1227/Ser1345	Regulates cell size and cell survival under energy starvation condition	24
	Raptor	Ser722/Ser792	Mediates a metabolic checkpoint	25
	ULK1	Ser317/Ser555/Ser777	Induces autophagy under glucose starvation	26,27
	PRAS40	Thr246	Regulates the activity of mTORC1	28
	FOXO3a	Ser413/Ser588	Transcriptionally represses SKP2	29
LKB1	AMPKα	Thr172	Mediates the prolonged and adaptive activation of AMPK following energy stress	30
CaMKKβ	AMPKα	Thr172	Activates AMPK in response to increase in cellular Ca^{2+}	31
TAK1	AMPKα1/2	Thr183/Thr172	Activates AMPKα1/2	32
mTORC1	ULK1	Ser757	Disrupts the ULK1–AMPK interaction	26
	DAP1	Ser3/Ser51	Inhibits autophagy indirectly	33
	AMBRA1	Ser52	Inhibits autophagy indirectly	33,34
	TFEB	Ser142	Inhibits autophagy indirectly	33,34
ULK1	Beclin-1	Ser14	Induces autophagy	16
	ATG9	Ser14	Promotes ATG9 trafficking in response to starvation	35
	ATG4B	Ser316	Inhibits ATG4B activity and LC3 processing	36
	AMBRA1	Ser465/Ser635	Regulates dissociation of AmpRa1–Vps34–beclin-1 from the dynein complex	37
	Raptor	Ser855/Ser859/Ser792	Inhibits mTORC1 during starvation	38
	ATG13	Ser318	Promotes its release to damaged mitochondria	39
PKCα	ULK1	Ser423	Prevents autolysosome formation	40
DAPK1	Beclin-1	Thr119	Liberates beclin-1 from BCL-2 and BCL-XL	41
DAPK3	Beclin-1	Ser90	Regulates autophagy in skeletal muscle	42
DAPK2	Raptor	Ser721	Modulates mTORC1 activity and autophagy level	43
JNK1	BCL-2	Thr69/Ser70/Ser87	Dissociates BCL-2 from beclin1	44
PIM2	TSC2	Ser1798	Relieves the suppression of TSC2 on mTORC1	45
	BAD	Ser112	Prevents dissociation of BCL-2 from beclin-1	46
GSK-3	Raptor	Ser859	Regulates mTORC1	47
LRRK2	EndoA	Ser75	Modulates the membrane curvature	48
PINK1	Parkin	Ser65	Leads to the aggregation of parkin from cytoplasm to damaged mitochondria	49

– Not applicable.

3.2. *AMPK/mTOR/ULK1*

Another major mTOR-related signaling pathway is the AMPK–mTOR pathway. AMPKs are a type of evolutionarily conserved serine/threonine protein kinases which are composed of 13 members[51]. The AMPKs serve as master sensors of cellular energy status that is of great significance in energy homeostasis. AMPK is activated by a low energy state and its role in autophagy initiation has been clearly shown. On the one hand, the activation of AMPK can phosphorylate TSC2 and subunit Raptor at Ser792 of mTORC1[24,25]. On the other hand, AMPK initiates autophagy by direct phosphorylation of ULK1 at Ser317 and Ser777 under glucose starvation[26]. Additionally, a new signal axis of AMPK–SKP2–CARM1 has been discovered which can regulate autophagy induced by nutrient starvation[29]. The regulation mechanism of AMPK is extremely complex. The $\alpha\beta\gamma$ trimeric AMPK complexes are allosterically regulated mainly by the ratio of AMP/ATP[52]. AMPK is also subject to the regulation by upstream kinases like serine/threonine-protein kinase stk11 (LKB1) and calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ). Another kinase, transforming growth factor activated kinase-1 (TAK1) has also been identified, which is able to activate Snf1 kinase in yeast and phosphorylates AMPKα1/2 at Thr183/Thr172 *in vitro*[53–55].

mTOR, known as the mammalian target of rapamycin (also known as FRAP), serves as a core component of two structurally and functionally distinct protein complexes, mTORC1 and mTORC2. The former senses and responds to fluctuations in the levels of intra- and extracellular nutrients, primarily amino acids as well as growth factor signaling, cellular energy and oxygen levels. It has been validated as a primary node in coordinating the respective anabolic and catabolic processes in response to various stresses. In mammals, mTORC1 regulates autophagy under nutrient-rich conditions through directly phosphorylating and suppressing ULK1 complex, which is required for autophagy initiation[23,56,57]. Additionally, under nutrient-deprived conditions, mTORC1 also can negatively regulate autophagy *via* phosphorylating autophagy/beclin-1 regulator 1 (AMBRA1) at Ser52 and phosphorylating DAP1 at Ser3 and Ser51[33,58]. Other work has shown a new link between mTORC1 and autophagy regulation: mTORC1 directly phosphorylates the transcription factor EB (TFEB) at Ser142, which is required for lysosome biogenesis[59,60]. mTORC2 was reported to indirectly suppress autophagy through AKT/mTORC1 signaling axis activation[61,62].

As another pivotal node in autophagy[14,63], ULK1 is a component of the ULK1–ATG13–FIP200–ATG101 complex (ULK complex) required for autophagy induction. Specifically, under starvation conditions, as mentioned above, ULK1 is directly and indirectly activated by AMPK and subsequently phosphorylates beclin-1 on Ser14 and activates Vps34 lipid kinase[16]. The latter is essential for full autophagic induction. Besides, ULK1 can

phosphorylate ATG9 at Ser14 synergistically with the proto-oncogene tyrosine-protein kinase SRC, thus promoting translocation of ATG9-positive vesicles to the autophagy initiation sites[35]. Also, ULK1 phosphorylates ATG4B at Ser316, and ATG4B phosphorylation is responsible for the conversion of pro-LC3 to LC3-I and LC3-II back into LC3-I[36]. Additionally, ULK1 also can modulate autophagy *via* phosphorylation of other substrates like AMBRA1 and Raptor[37,38]. Apart from phosphorylation of ULK1 by mTOR and AMPK, ULK1 activity is also fine-tuned by additional mechanisms and other kinases (*e.g.*, PKCα and P38). PKCα phosphorylates ULK1 at Ser423 and prevents autolysosome formation without a direct change of ULK1 activity[40]. P38α-dependent phosphorylation of ULK1 triggered by lipopolysaccharide (LPS) leads to the inhibition of ULK1, preventing it from binding to the downstream effector ATG13. This pathway was shown to eventually reduce autophagy in microglia[64].

3.3. *Kinases regulate autophagy via beclin-1 and its interactomes*

Beclin-1 (the mammalian orthologue of yeast Atg6) is another key node of autophagy regulation in mammalian cell which interacts with several interactomes (such as ATG14L, UVRAG, BIF-1, Ambra1, HMGB1 and PINK) to regulate Vps34 and promote formation of PI3KIII core complexes, thereby inducing autophagy[65,66]. Although not a kinase itself, beclin-1 and its interactomes are regulated by several kinase-mediated phosphorylation reactions in autophagy. The section below describes how other kinases regulate autophagy though beclin-1 and its interactomes, such as DAPKs and JNK1.

Death-associated protein kinase (also known as DAPK), a Ca^{2+}-calmodulin regulated kinase, was reported to stimulate autophagy and membrane blebbing by binding to LC3. Specifically, DAPK1 phosphorylates beclin-1 on Thr119 at the BH3 domain, thus liberating beclin-1 from BCL-2 and BCL-XL, and, in turn, promoting autophagy[41,67]. Also, DAPK1 stimulates autophagy *via* ARF-dependent accumulation of P53[68]. DAPK3 is also reported to control autophagy by directly phosphorylating beclin-1 at Ser90 in skeletal muscle tissues, providing an enhanced understanding for the mechanism through which metabolism and autophagy are linked[42]. Compared to DAPK1 and DAPK3, DAPK2 was shown to phosphorylate raptor at Ser721 to modulate mTORC1 activity and autophagy levels under stress and steady-state conditions[43].

JNK1 controls autophagy *via* phosphorylating the anti-apoptotic protein BCL-2 at residues Thr69, Ser70 and Ser87 of the non-structured loop which causes dissociation of BCL-2 from beclin-1[44]. Moreover, this process has also been implicated in the induction of apoptosis. A model has been proposed to explain how cells balance the interaction between autophagy and apoptosis *via* JNK1-mediated BCL-2 phosphorylation[69].

3.4. *Other kinases in autophagy regulation*

Previous work has shown that serine/threonine-protein kinase PIM-2 (one of three PIM kinases) is involved in autophagy regulation by activation of the mTOR pathway. Aberrant PIM-2 expression has been observed in a variety of malignancies. Evidence showed that PIM2 can directly phosphorylate TSC2 on Ser1798 and relieve the suppression of TSC2 on mTORC1[45]. Furthermore, PIM2 promotes autophagy and PIM2-suppression decreases the autophagic response and prevents dissociation of BCL-2 from beclin-1 and enhancing lysosomal acidification[46]. Other work demonstrated that phosphorylation of hexokinase-II by PIM2 was required for autophagy during glucose starvation[70].

GSK-3, an ubiquitously expressed serine/threonine kinase, was initially discovered as a regulator of glycogen synthesis, has also been found to be involved in autophagy modulation. In MCF-7 cells, GSK-3α/β overexpression activates mTORC1 and inhibits autophagy *via* phosphorylating Ser859 on raptor, resulting in reduced p70S6K1 and ULK1 phosphorylation along with increased autophagic flux[47]. In a prostate cancer cell model, GSK-3β was found to control autophagy by modulating the LKB1−AMPK pathway[71]. Thus, GSK-3β inhibition caused a rapid cellular ATP decline, and subsequently LKB1-depedent AMPK activation and mTOR pathway inactivation were associated with autophagy induction.

ERK1 (also known as p44MAPK or MAPK3), one isoform of extracellular signal-regulated kinase (ERK) that belongs to mitogen-activated protein kinases (MAPKs), plays a role in autophagic regulation in various tumor cells[72,73]. ERK1 was phosphorylated and activated to regulate autophagy *via* the RAS−RAF−MEK axis[74]. Another lab reported that non-classic activation of MEK/ERK can also modulate beclin-1 expression to stimulate autophagy[75]. Acute activation of MEK/ERK causes cytoprotective autophagy by inhibition of either mTORC1 or mTORC2 with a moderate increase of beclin-1 expression. However, prolonged activation of MEK/ERK leads to dual inhibition of mTORC1 and mTORC2, with a definitive increase in beclin-1 expression and cytodestructive autophagy.

Leucine-rich repeat kinase 2 (LRRK2), a member of the leucine-rich repeat kinase family, has also been implicated in autophagy. LRRK2 regulates autophagy by phosphorylating EndoA at Ser75, which in turn modulates the membrane curvature, thus controlling the recruitment of the autophagy machinery to the nascent autophagosome[48]. Besides, prolonged LRRK2 kinase inhibition increases phosphorylation on Ser758 ULK1 *via* an unknown regulatory feedback loop[76]. Another lab found that membrane-associated LRRK2 inactivated beclin-1 and consequently inhibited autophagy, supporting LRRK2 as a primary inhibitor of autophagy[77].

PTEN-induced putative kinase 1 (PINK1) and parkin RBR E3 ubiquitin protein ligase (Parkin/PARK2) mediate mitophagy, which can clear dysfunctional mitochondria. Mounting evidence has shown that PINK1 acts as a gatekeeper of mitochondrial quality control[78]. PINK1 directly phosphorylates Parkin at a highly conserved Ser65[79], leading to the aggregation of parkin from cytoplasm to damaged mitochondria, and finally clears the organelles depending on mitophagy[49].

4. Kinase inhibitors/activators for autophagy inhibition and induction

Modulation of the autophagy-related kinases discussed above has the potential to modify autophagy processes. Of note, autophagy inhibition and induction are achievable by small-molecule kinase inhibitors/activators. Therefore, in this following section, we review some kinase modulators applied to autophagy induction (Table 3 [31,76,80−116]) and inhibition (Table 4[117−124]).

Table 3 Kinase inhibitors/activators for autophagy induction.

Name	Mechanism	Cell line	Disease	Clinical status	Ref.
GDC-0941	PI3K inhibition	MCF-7, T47D and ZR75-1 cells	ER^+ breast cancer	Phase 1	80
Taselisib	PI3K inhibition	Human KPL-4 breast cancer cell	Advanced solid tumors	Phase 3	81
PX-866	PI3K inhibition	LNZ308 and LN229 cells	Glioblastoma	Phase 1	82
PKI-587	PI3K/mTOR inhibition	A431-CR and FaDu-CR cells	Breast cancer, non-small cell lung cancer, ovarian cancer, etc.	Phase 1/2	83
BEZ235	PI3K/mTOR inhibition	786-0 and Caki-1 cells	Metastatic renal cell carcinoma	Phase 1	84,85
PF-04691502	PI3K/mTOR inhibition	A549 cells	Non-small cell lung cancer	Phase 1	86
Perifosine	AKT inhibition	T98G and U373MG cells	Glioblastoma, anaplastic astrocytoma, mixed glioma, etc.	Phase 2	87,88
MK2206	AKT inhibition	UL cells	Colon mucinous adenocarcinoma, colon signet ring cell adenocarcinoma, rectal mucinous adenocarcinoma, etc.	Phase 2	89
BI-69A11	AKT inhibition	HT-29 cell	–	–	90
Metformin	AMPK activation	EC109 cells	Diabetes mellitus type 2	Phase 4	91
Salicylate	AMPK activation	HEK-293 cells	–	–	31
Hernandezine	AMPK activation	HeLa cells	–	–	92
Simvastatin	AMPK activation	Mouse coronary arterial myocytes	Coronary arterial myocytes	Phase 1/2	93
A-769662	AMPK activation	U373 cells	–	–	94
Rapamycin	mTOC1 inhibition	SK-N-SH and SH-SY5Y cells	Primitive neurotodermal tumor and mast cell leukemia	–	95,96
AZD8055	mTOC1 inhibition	H838 and A549 cells	Glioblastoma multiforme, advanced hepatocellular carcinoma, advanced solid malignancies, etc.	Phase 1	97
PP242	mTOC1 inhibition	HeLa cells	–	–	98
RapaLink-1	mTOC1 inhibition	MCF-7, RR1 and RR2 cells	–	–	99
LYN-1604	ULK1 activation	MDA-MB-231 cells	Triple negative breast cancer	–	100
BL-918	ULK1 activation	SH-SY5Y cells	Parkinson's disease		101
JNK-IN-8	JNK1 inhibition	Primary hepatocytes and primary epithelial cells	–	–	102
HJ-PI01	PIM2 inhibition	MDA-MB-231 cells	–	–	103
SMI-4a	PIM2 inhibition	A375 and G361 cells	–	–	104
SGI-1776	PIM2 inhibition	MM.1S cells	Relapsed/refractory leukemias	Phase 1	105
AZD1208	PIM2 inhibition	Primary chronic lymphocytic leukemia cells	Primary chronic lymphocytic leukemia	Phase 1	106
SB216763	GSK3β inhibition	Human pancreatic cancer cells	–	–	107
CHIR99021	GSK3 inhibition	Human pancreatic cancer cells	–	–	108
TDZD8	GSK3β inhibition	PC-3 cells	–	–	109
9-ING-41	GSK3 inhibition	PC-3 cells	Lymphoma pancreatic cancer	Phase 1/2	110
SCH7 72984	ERK1 inhibition	HPAC and PANC-1 cells	–	–	111
BVD-523	ERK1 inhibition	HPAC and PANC-1 cells	Advanced solid tumors	Phase 1/2	111,112

Table 3 (*continued*)

Name	Mechanism	Cell line	Disease	Clinical status	Ref.
GDC-0994	ERK1 inhibition	Epithelial Caco-2 and HT-29 cells	–	–	113
LRRK2-IN-1	LRRK2 inhibition	H4 astroglioma cells	–	–	76
GSK2578215A	LRRK2 inhibition	SH-SY5Y cells	–	–	114
SB202190	MAPK inhibition	HT29 cells	–	–	115
SB203580	MAPK inhibition	HCC cells	–	–	116

– Not applicable.

Table 4 Kinase inhibitors for autophagy inhibition.

Name	Mechanism	Cell line	Disease	Clinical status	Ref.
LY294002	PI3K inhibition	Mel Z and Mel IL cells	Neuroblastoma	Phase 1	117
Wortmannin	PI3K inhibition	CHO cells	–	–	118
3-MA	PI3K inhibition	Mouse embryonic fibroblast	–	–	119
Dimeric quinacrines	mTORC1/autophagy dual inhibition	PANC1, A375P and C8161 cells	–	–	120
SBI0206965	ULK1 inhibition	PDAC cell lines	–	–	121
MRT67307	ULK1 inhibition	HeLa cells	–	–	122
MRT6892	ULK1 inhibition	HeLa cells	–	–	122
ULK-101	ULK1 inhibition	U2OS cells	–	–	123
SP600125	JNK inhibition	HT29 cells	–	–	124

4.1. Kinase inhibitors/activators for autophagy induction

As discussed above, PI3K inhibition can lead to autophagy induction. In a case of ER^+ breast cancer treatment, autophagy was induced by the PI3K inhibitor GDC-0941[80]. Taselisib and PX-866 were also reported to induce autophagy respectively in ovarian cancer cells and in glioblastoma cells[81,82,125]. Additionally, since the catalytic subunit structure of mTOR resembles that of PI3K, many PI3K inhibitors can also potently inhibit mTOR. After the combination treatment of the dual PI3K/mTOR inhibitor PKI-587 with cetuximab, A431-CR and FaDu-CR cell lines displayed an increased Beclin-1 expression and a decrease in p62 levels[126]. Consistently, the dual PI3K/mTOR competitive inhibitor BEZ235 induced autophagy in human glioma and hepatocellular carcinoma cells[84,127]. Another study showed that dual PI3K/mTOR inhibitor PF-04691502 induced autophagy in non-small-cell lung cancer cell lines *in vitro*, demonstrated by upregulated LC3-II and beclin-1 expression[86]. These dual inhibitors were used to prevent drug resistance effectively in curing various cancers like breast cancer, T-cell acute lymphoblastic leukemia, gastric cancer and lymphomas[83,85,128].

As an AKT inhibitor, the phosphatidylinositol analog perifosine[87] induces protective autophagy and upregulation of ATG5 in human chronic myelogenous leukemia cells[88]. The allosteric inhibitor MK2206 was reported to induce autophagy[89], which showed potent activity against multiple cancers in clinical trials[129,130]. In addition, a novel AKT inhibitor BI-69A11 was reported to induce autophagy at earlier time point through the inhibition of AKT/mTOR/p70S6 kinase pathway[90].

Autophagy can also be induced by AMPK activation. To date, more than 100 different natural plant products and derivatives originating from traditional medicines can activate AMPK by several different mechanisms. Metformin and salicylate are the most successful and widely used AMPK activators[31,91], both of which commonly appear in the autophagy-related research for AMPK activation[131–133]. Also, hernandezine, a novel AMPK activator, was reported to induce autophagic cell death in drug-resistant cancers such as HeLa cells[92]. Significant progress has been made in the development of direct small molecule AMPK activators over the last few years. For instance, an AMPK activator, simvastatin increases autophagy in coronary arterial myocytes *via* inhibiting the RAC1-mTOR pathway[93]. Another study has found that activation of AMPK by A-769662 led to an increased expression of the autophagosomal markers LC3 and P62/SQSTM, suggesting an efficient induction of autophagy[94]. Conversely, AMPK inhibitor dorsomorphin was reported to induce autophagy in T24 cells *via* AMPK-independent inhibition[134].

It is well established that mTORC1 inhibition can induce autophagy. For example, rapamycin and its derivatives are well known as autophagy inducers by inhibiting mTORC1 and are widely used in autophagy related research[95]. Furthermore, many other mTOR inhibitors have been developed. In a variety of cancer cells like H838 and A549 cells, AZD8055 can induce the formation of acidic vesicles associated with LC3, consistent with the induction of autophagosome formation, suggesting an activation of autophagic flux[97]. The ATP competitive mTOR inhibitor pp242, a stronger autophagy inducer than rapamycin, is able to induce lysosomal activation *via* blockade of mTORC1 activity[98]. RapaLink-1, a third-generation mTORC1 inhibitor, overcomes

resistance to existing first- and second-generation inhibitors through exploiting the unique juxtaposition of two drug-binding pockets to create a bivalent interaction that allows inhibition of resistant mutants[99]. A recent study also found that RapaLink-1 can activate autophagy, but the specific mechanism is unknown[135]. Additionally, LYN-1604, the first ULK1 agonist, can induce cell death associated with autophagy in MDA-MB-231 cells by interfering with the formation of the ULK complex. This drug showed potential for good therapeutic effects on TNBC[100]. BL-918, a potent activator of ULK1, induced autophagy *via* the ULK complex, and displayed a cytoprotective effect on MPP^+-treated SH-SY5Y cells, which may be a candidate drug for Parkinson's disease (PD) treatment[101].

Treatment with the JNK1 inhibitor JNK-IN-8 increased the accumulation of LC3B-II following lysosomal inhibition and reduced the accumulation of SQSTM1, demonstrating increased autophagic flux[102]. Additionally, among the few PIM inhibitors identified by far, HJ-PI01 was able to induce autophagic death in MDA-MB-231 cells[103]. The pan-PIM inhibitor SMI-4a also induced autophagy through inhibition of the PI3K/AKT/mTOR axis in melanoma cells[104]. Induction of autophagy was also interrupted by the PIM inhibitor SGI-1776 in MM cell lines and bone marrow $CD138^+$ cells[105]. The PIM kinase inhibitor AZD1208, inhibits protein translation by decreasing phosphorylation levels of eukaryotic translation initiation factor 4E-binding protein (4E-BP1) and induces autophagy in primary CLL cells[106].

Modulation of other kinase targets can also alter autophagy. Inhibition of GSK3β with SB216763 promotes autophagy induced by starvation *in vitro*[107]. Also, GSK3 inhibition with SB216763 or CHIR99021 induces an autophagic response in human pancreatic cancer cells[108]. GSK-3β suppression by TDZD8, a non-ATP competitive small molecule, promotes autophagy during serum starvation[109]. In renal cancer cells, the GSK-3 inhibitor 9-ING-41 leads to AMPK activation and autophagy induction[110]. In a recent study, the ERK1-selective inhibitor SCH7 72984 (an analog of the clinical candidate MK-8353) and the clinical candidate BVD-523 were reported to elevate autophagic flux. These results suggest that concurrent blockade of both ERK and autophagic processes that are upregulated in response to ERK inhibition, may be an effective approach for treating pancreatic ductal adenocarcinoma[111]. GDC-0994 is also an ERK inhibitor, which suppressed ERK phosphorylation, and thus inhibiting p-mTOR and activating autophagy[113]. The LRRK2 inhibitor LRRK2-IN-1 was reported to stimulate autophagy in a non-canonical fashion. This mechanism was independent of mTOR and ULK1, but dependent upon the activation of class III PI3-kinase[136]. GSK2578215A also induces protective autophagy in SH-SY5Y cells[114]. Other p38a inhibitors like SB202190 and SB203580 can induce autophagy *via* different mechanisms; the former acts *via* p38a blockade[115], whereas the latter activates AMPK and DAPK[116].

4.2. *Kinase inhibitors/activators for autophagy inhibition*

In recent years, some small-molecule kinase inhibitors have been used to inhibit autophagy. For instance, Wortmannin, LY294002 and 3-MA, the first classical generation non-selective pan-PI3K inhibitors[117–119], are known as autophagy suppressors. Dimeric quinacrines (DQ) were reported to concurrently inhibit both mTORC1 and autophagy as a unified approach to targeting lysosomes degradation and growth signaling roles[120]. These differ from normal mTOR inhibitors which induce autophagy. ULK1 inhibitors can also inhibit autophagy. For example, compound SBI0206965 exhibits good inhibitory potency of ULK1 and can be used as an autophagy inhibitor[121]. MRT67307 and MRT6892 share the same scaffold and the ability to inhibit ULK1 and ULK2 *in vitro* and subsequently block autophagy in cells[122]. Another inhibitor, ULK-101, exhibits a similar degree of ULK1 inhibition as MRT6892, and suppresses autophagy induction and autophagic flux in response to different stimuli such as nutrients, including amino acids and growth factors[123]. In addition, JNK inhibitors have also been used for autophagy inhibition. For instance, the first reported effective JNK inhibitor SP600125 inhibited autophagy *via* reduction of beclin-1[124].

5. Conclusions and prospects

Both cell survival and cell death are regulated by autophagy. Precise positioning of autophagy in the specific disease context confers a rational basis for deciding the proper direction for modulation of autophagy. For example, numerous previous studies have indicated autophagy determines the fate of cancer cells depending on the type, stage and genetic context[137–141]. Autophagy inducers like rapamycin contribute to lower oncogenic risk caused by deficiencies in autophagy function required for the initiation of cancer[142]. However, when cancer is established, autophagy inhibition is needed to cope with the pro-survival effects of autophagy. For instance, a combination of autophagy inhibitors can sensitize tumor cells to metabolic stress induced by chemotherapy (*e.g.*, angiogenesis inhibitors). Furthermore, as in the case of neurodegenerative disorders, activation of moderate protective autophagy for degradation of accumulated misfolded proteins serves as a reasonable therapeutic approach[143,144]. Thus the use of autophagy inducers is likely to have benefit in this situation.

Nearly all signal transduction processes are linked to phosphate transport cascades, suggesting that a true physiological response (autophagy and beyond) can be induced by changing the activity of kinases. Indeed, autophagy-related kinases are commonly multi-functional. For instance, mTOR, the gatekeeper of autophagy, is also implicated with cell growth, cell metabolism and protein synthesis[145]. Additionally, ULK1, as the autophagy initiator, not only regulates multiple steps in the autophagy pathways, but also modulates cellular processes such as ER-to-Golgi trafficking and axonal growth, as well as PARP1 activation related to programmed cell death[146]. Nevertheless, there is no doubt of the essential regulatory roles of these kinases in autophagy. In particular, mTOR and ULK1 play pivotal roles in autophagy induction and their kinase activities are closely associated with autophagy initiation. Although other autophagy-related kinases, such as AKT, PI3K, and AMPK, mainly act as important regulators of cell proliferation and metabolism, inhibition/activation of these kinases are also significant to autophagy signal transduction[147,148]. Whether other kinases (not emphasized in this review) have the potential to regulate autophagy and become drug targets need to be validated by future studies. Furthermore, as for the unknown side effects caused by kinase inhibition/activation, it has not been determined whether these autophagy-related kinases can be used as a breakthrough point of autophagy intervention.

Despite the potential therapeutic benefits of autophagy-related kinase inhibitors/activators in animal models of disease, their clinical development into useful drugs will be challenging. First of all, due to the lack of organ-specificity, utilization of

autophagy-related kinase inhibitors/activators may lead to unwanted and uncontrolled side effects, unless the side effects are tolerable for the duration of intended use. Considering the protective effects of autophagy on neurons, it is sensible to improve the brain-specificity of autophagy-related treatments for neurodegenerative diseases. To achieve the goal of organ-specificity, different delivery strategies and photodynamic chemotherapy are proposed. Additionally, these small-molecule autophagy-related kinase inhibitors/activators share the problems of resistance and target selectivity[149], as known for the common kinase drugs. The occurrence of drug resistance of autophagy-related kinase inhibitors/activators is a formidable obstacle to surmount because of factors such as gene mutation, kinase up-regulation, and compensatory mechanisms and bypass effects. New therapies are needed to approach these limitations and treat the evolving diseases, especially cancer. For instance, RapaLink-1, a third-generation mTOR inhibitor, exploits the unique juxtaposition of two drug-binding pockets to create a bivalent interaction that reverses the resistance to existing first- and second-generation inhibitors. So far, several strategies have been proposed to solve the problem of target selectivity. First, compared to target ATP sites shared in kinase family, targeting allosteric sites of kinases takes distinct advantages like enhanced specificity, reduced side effects, and lower toxicity. Another approach is covalent targeting of kinases, used to design reversible and irreversible covalent kinase inhibitors/activators with a better pharmacokinetic characteristics[150].

At present, mounting innovations contribute to a better use of small-molecule kinase inhibitors/activators. The popular proteolysis targeting chimeras (PROTAC) approach provides a new profile for the application of autophagy-related kinase inhibitors/activators. And new computational methods such as available 3D-e-Chem-VM help to predict selectivity profiles. Meanwhile, artificial intelligence holds great promise in discovery, transformation and application of kinase agents. Based on the current trends discussed above, we believe that autophagy-based kinase targeting therapy presents a fascinating direction of autophagy research and is a promising beneficial therapeutic approach.

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Author contributions

Lan Zhang, Bo Liu, and Liang Ouyang were responsible for the conception and design of the review. Honggang Xiang, Jifa Zhang and Congcong analyzed the literatures, summarized the results and drafted the manuscript. Lan Zhang, Bo Liu, and Liang Ouyang revised the manuscript. All authors have read and approved the final manuscript.

Conflicts of interest

The authors claim that the researchers in this study have no conflict of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2019.10.003.

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