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Review Article

ULK1/2 Inhibitor: Essential Component of Autophagic Cell Death Machinery

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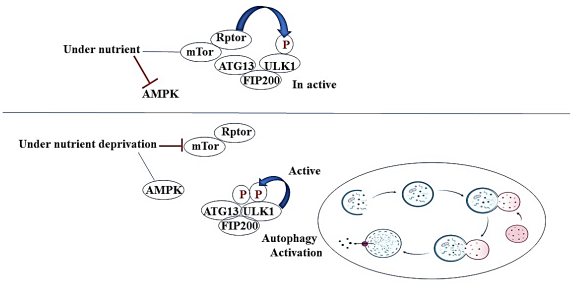
ABSTRACT

The effectiveness of selective drug therapy for cancer patients has gained much attention from academics and society. However, the rapid development of the drug resistance gained is becoming a significant challenge. As an essential catabolic and homeostatic process, autophagy plays a vigorous role in the degradation or recycling of proteins and cellular components, by which eukaryotic cells recycle or degrade their internal constituents through the machinery of membrane trafficking. Therefore, under traumatic conditions autophagy provides the cells with a safe supply of biomolecules and energy to maintain homeostasis. Deregulation of autophagy pays to tumor genesis, tumor-stromal interactions, and resistance to cancer therapies. Outcome of these interactions between plants and viruses, autophagy performs a vital role in regulating immune-related cell death, antiviral defense, and viral pathogenesis. This study explores the role of autophagy in drug resistance by identifying the mechanism by which autophagy is elaborate in drug resistance, focusing on its mode of action and validation as a potential therapy.



Keywords: Autophagy mechanism; Cancer; ULK1/2 inhibitors

INTRODUCTION

A specialized organ within cells known as the lysosome contains hydrolytic enzymes discovered in the 1960s by Nobel Laureate Christian de Duve. During the same era of discoveries, Novikoff and colleagues demonstrated through electron microscopy that lysosomes contain cytoplasmic components (thick intracellular bodies).[1] As an outcome of these pioneering discoveries, cytoplasmic components can be released to lysosomes for degradation. The autophagy progression is characterized by double-membrane vesicles. The bulk cytoplasm of these autophagosomal vesicles was identified by morphological characterization.[2] However, this entails that autophagy contributes to a non-selective

degradation of cytoplasmic material; de Duve predicted that there also had to be a controlled and selective form of this way. The mechanisms underpinning autophagosome biogenesis and its regulation remained largely unknown until the 1990s, but electron microscopy allowed for the morphological assessment of autophagy. Genetic screens were invented by Schekman and colleagues to identify yeast proteins secretion genes. Maybe this invention stimulates Yoshinori Ohsumi[3] and co-workers for developing a genetic screen to identify autophagy-defective mutants in *Saccharomyces cerevisiae*. The revolutionary discoveries from both investigators were honored with Nobel Prize in Physiology or Medicine. First for Schekman followed by Ohsumi in 2013 & 2016 respectively for their characterization of fundamental cellular transportation processes.[2]

According to Yoshinori Ohsumi, autophagy has a significant role in biology and many diseases, as well as in cancer. The phrase "autophagy," translated literally as "eating oneself," (from the Greek, "auto" oneself, "phagy" to eat) describes three mechanistically distinct devices by which the lysosomal hydrolases carry cellular material to the lysosome for degradation.[4–6] The macromolecular precursors such as nucleic acid, amino acids, free fatty acids, carbohydrates, proteins, triglycerides to nucleosides, etc. are then recycled to assemble new macromolecules or consumed as fuel to metabolic pathways.[7] Research exposed that autophagy machinery is likewise elaborate in non-degrading processes (e.g., in cellular secretion and in controlling signal transduction pathways.[4] When circumstances of nutrient hunger are met, autophagy is strongly induced and results in major cytoplasmic component degradation (proteins, organelles) whose building blocks are used for the supply of energy and the synthesis of components necessary for survival under nutrient hunger situations.[8] Throughout autophagic cells, the total intracellular supply of amino acids is significantly reduced, contributing to the cell's failure to synthesize proteins that are an important for survival.[9] Parallel findings were detected in mutant cells where autophagy still functioned generally, but amino acid efflux to the cytosol was impaired owing to the deficiency of the putative vacuolar amino acid transporter Atg22.[10] Furthermore, autophagy serves other functions and also being essential to cellular housekeeping because it removes depleted, obsolete, or unwanted components. Autophagy may help as an anti-aging mechanism in this manner.[11] Help cell remodeling or contribute to cellular protection against pathogens throughout development.[12]

The ULK1 kinase inhibits autophagy, which may be castoff to treat cancer. ULK1, another closely connected kinase, has been studied and its inhibitors identified, but slight is recognized about ULK2. There were numerous effective ULK1/2 inhibitors discovered after screening a library of pre-clinical and clinical compounds focused on kinases, as well as upright connection between inhibitor binding behavior and both ULK kinases.

There is a kinase domain at the N-terminus, a serine-proline-rich region at the C-terminus, and an interacting domain at the C-terminus of ULK. The serine-proline-rich region of ULK1 is phosphorylated by mTORC1 and AMPK, both of these are negatively and positively regulated by the protein. In the C-terminal side chain, there are two microtubule-interacting and transport (MIT) domains and they form a scaffold that connects ULK1, ATG13, and FIP200 to induce autophagy. Two three-helix. bundles are arranged within the early autophagy targeting/tethering (EAT) domain at its C-terminus. MIT domains also mediate interactions with membranes. Among the N and C termini of ULK1, there is a large positively charged activation loop. It may be involved in kinase activity and substrate recognition. In both the C-terminal and N-terminal domains, ULK1 and ULK2 have significant homology. In the following sections, we outline the current knowledge regarding the regulation of autophagy in cancer and its impact on various processes that protect against malignant disease, and in this review, we target ULK inhibitor's roles in the autophagic process.

Autophagy is essential in eukaryotic cells for homeostasis (a highly regulated process). Autophagy's diverse cytoplasmic targets helps to treat various human diseases, counting neurodegenerative disorders, heart disease, and cancer.[13,14] Autophagy sequesters cytoplasmic materials by the autophagosome (which elongates & surrounds a portion of the cytoplasm to form a dual-membraned structure) and transferred to the lysosome for digestion.[14,15] Various phases of autophagy are induction, isolation membrane formation (phagophore), autophagosome creation & maturation, and ultimately, fusion with a late endosome or lysosome.[14,16,17] The creation of autophagosomes from isolating membrane is a highly regulated and complex process that involves diverse types of protein and gene complexes (Figure 1).[18] The macroautophagy is the dominant form of autophagy among all forms. It is responsible for the degradation under tension conditions of both soluble proteins and organelles.[19]

MECHANISM OF AUTOPHAGY

The autophagic process contains of numerous stages such as initiation (isolation of membrane), nucleation (Elongation), maturation, fusion, and degradation. The capacity to reprocess macro molecules by autophagy gives cells a significant advantage under stressful circumstances such as lack of carbohydrates, oxidative stress, oxygen deprivation, ER stress, metabolic stress, etc.[17] Many genes and proteins are involved in the machinery of autophagy, including FIP200, ULK1 & 2, BECN1, BCL-2, ATG 13, UVRAG, AMBRAN1, VPS34, BCL-XL, VPS34, ATG14, VPS15, ATG101, RUBICON are helps in the process of induction and nucleation. In elongation process helps ATG3, ATG5, LC3-PE, ATG16, ATG7, ATG12, ATG48, ATG10 proteins. And in the last fusion process STX17, LAMP2, ATG2A,

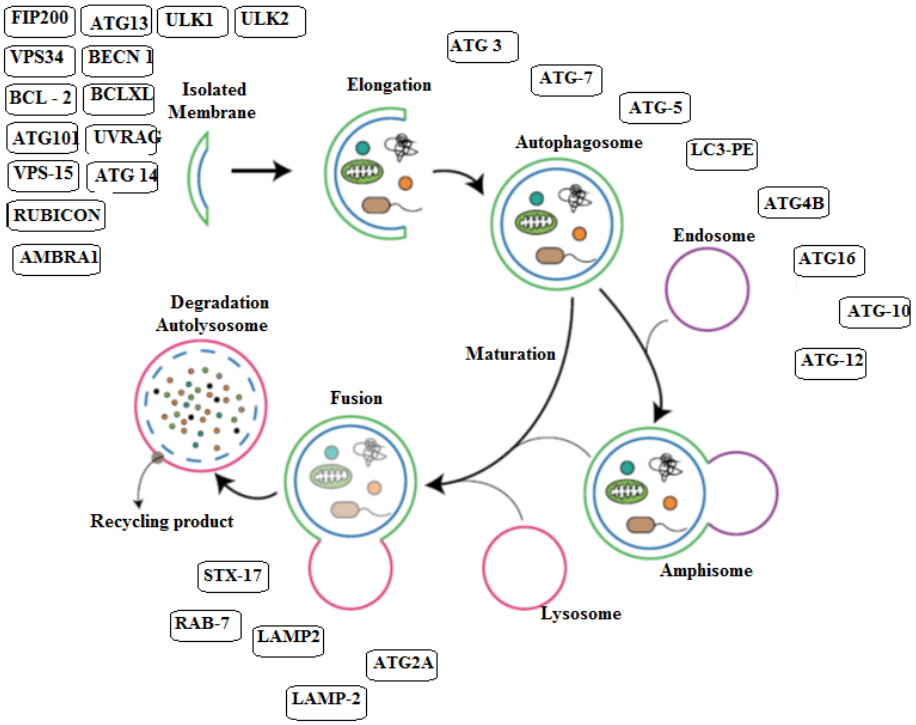


Fig. 1: Mechanism and Regulation of autophagy process

RAB7, ATG2b proteins are involved. All are playing a grave role in the whole mechanical process of autophagy.

Initiation Stage

A small fragment initiates the various steps of autophagy, components of proteins, and modulation points. In the beginning of an auto-phagosome, the mammalian target of Rapamycin (mTOR) delivers a variability of signals and activates the Unc-51like kinases 1 and 2 (ULK1 and ULK2, the two mammalian homologs of ATG1). mTOR occurs as two separate complexes in mammals: mTOR complex1 (mTORC1) and mTOR complex2 (mTORC2).[20–22] Cellular energy homeostasis is regulated by mTOR complexes that integrate anabolic and catabolic pathways. Under nutrient-rich conditions, mTORC1 complexes suppress autophagy by inactivating the ULK1/2 complex, which contains ULK1 or ULK2 kinase, ATG13 (family interacting protein of 200 kDa), FIP200 (the mammalian homolog of ATG17) and ATG101 (also called C12orf44)(21,22). When nutrient deprivation occurs, ULK1/2 complex is activated. This activation leads to triggering autophagy via class III phosphatidylinositol 3-kinase (PI3K) complex (PIK3C3, also known as vacuolar protein sorting 34 or Vps34 which comprises VPS34, ATG14 L, VPS15-also known as PI3K regulatory subunit 4, PIK3R4).[21,23,24]

Nucleation stage

An autophagic membrane's nucleation is controlled primarily by a PI3K complex of class III phosphatidylinositol. The creation of phagophores has been structured by a number of novel proteins, including VPS34, Beclin-1, AMBRA1, and mATG9.[25,26] Beclin-1 interferes with autophagy by interacting with BCL2 during membrane nucleation. Counter-wise, distraction of this interaction allows Beclin-1 to bind with lipid kinase VPS34 and promote membrane nucleation.[27] VPS34-mediated phosphatidylinositol 3-phosphate (PtdIns3P) enzymatic generation offers a framework for phosphatidylinositol 3-phosphate (PI3P) binding domain-containing autophagy proteins, comprising WIPI1–4 and DFCP1.[21,28] 'Initiation complex' or 'class III PI-3K complex' activated by pre-initiation complex by phosphorylation of protein sorting by vacuolar protein 34 (VPS34) and Beclin-1.[25] Phosphatidylinositol-3-phosphate (PI3P) is directly controlled by the beginning complex. Cell pools the precursor phosphatidylinositol (PI) which is importantto isolate membrane nucleation.[29]

Maturation

The maturation and elongation of autophagosomes are instigated by two ubiquitin-like conjugation systems. First the ATG5–ATG12 complex conjugates with ATG16L1; second, microtubule-associated protein 1 light chain 3 (MAP1LC3, commonly called LC3).[21,22] At the membrane

nucleation site, accumulation of PI3P-binding domain-containing proteins leads to the necessary of additional ATGs, which are vital for elongation and culmination of the autophagosome membrane. ATG12 is a ubiquitin-like protein from covalent conjugation with ATG5 by the sequential action of the E1-like enzyme ATG7 and the E2-like enzyme ATG10 (ATG7 and ATG10 act as catalysts). The subsequent ATG5-ATG12 complex is engaged into the autophagic membrane and pays to its formation by cooperating with ATG16L1 and giving rise to the emergence of the ATG12-ATG5-ATG16L1 complex, which serves as an E3-like function to the second ubiquitin-like conjugation system.[21,22,30,31]

A second system involves conjugating LC3 with phosphatidylethanolamine (PE). Additionally, the USP10 and USP13 deubiquitination peptidases regulate a cascade of ubiquitination that results in cellular concentrations of initiation complex. The expansion of nascent precursor vesicles is based on the protein microtubule-associated autophagosome proteins 1A/1B light chain 3B (LC3). Key to this cycle is the conjugated form of the LC3 protein called LC3-II. The cytosolic form LC3-I is formed by cleavage of LC3 by ATG4, after conjugation to phosphatidylethanolamine (PE) LC3-I converts into LC3-II (an established marker to assay autophagic activity). The cleavage of LC3 allows revelation of the glycine residue at the C-terminal domain that chiefs to the creation of PE conjugation, making LC3-II the only protein stably associated with mature autophagosomal membranes during maturation.[21,31–33]

Fusion and Degradation

Lysosomes degrade autophagic vesicles once the autophagic membrane is formed. LC3-associated autophagosome proteins are delipidated and recycled before fusion.[34] Several SNARE proteins, including STX17 and WAMP8 and lysosomal integral protein LAMP2 and RAB proteins, play critical roles in autophagosome-lysosome fusion.[35] In autolysosomes, lysosomal proteases degrade cargo created by autophagosomes fusion with lysosomes. As the degradation products return to the cytosol, they are reused in different metabolic processes.[36] Autophagy has been revealed to be implicated in the pathogenic process of multiple diseases such as cancer, neurodegenerative, cardiovascular, metabolic, and immune diseases.[5]

AUTOPHAGY-MEDIATED CANCER REGULATION

Beth Levine's group proposed the link among autophagy and cancer in 1999, showing that deletions of the BECN1/ATG6 genes may lead to tumors in vitro[37] and in vivo.[38] Some studies have exposed that ATGs crosstalk with oncogenes and/or tumor suppressors. Nevertheless, accumulated data support the concept that the character of autophagy in malignant transformation is complicated and in a background and cell-type based way can have opposite consequences.[39] In addition to maintaining genomic stability, autophagy has been given away to be supportive for suppressing multi-stage cancer development by removing endogenous sources of reactive oxygen species (ROS); Maintenance of bioenergetics functions; oncogenic protein degradation and the initiation of immune response mechanisms against malignant transformation.[22] The autophagy progression involves many genes and proteins, and each has a diverse role at different stages. Detailed descriptions of all genes and proteins can be found in the Table 1.

ULK $\frac{1}{2}$

ULK1 is a protein kinase serine/threonine and Atg1 is a yeast mammalian ornithologist. There are five ULK1 homologues (ULK1, ULK2, ULK3, ULK4, and STK36 (serine/threonine kinase 36), however only ULK1 and ULK2 are known to be involved in classic autophagy signals.[64] In greatest cell lines, the deletion of ULK1 is adequate to disrupt autophagy; still, ULK2 is thought to act redundantly in this process. The necessity to knock out ULK1 and ULK2 in mice to display the same new mortality as other critical autophagy genes such as ATG5 and ATG7 demonstrates this redundancy.[65] As can be predictable for a protein kinase, the kinase activity of ULK1 is necessary for autophagy initiation: kinase-dead mutants of ULK1 and chemical inhibition of the enzymatic activity ULK1, result in autophagic flux blockage.[66] Unc-51-like protein kinase 1 (ULK1), Atg1's mammalian homology, is the preliminary protein during autophagy and the only serine/threonine kinase identified in the autophagic pathway.[67] Under certain stress circumstances, ULK1 activation via upstream signals such as AMPK and mTOR can cause autophagy. ULK1, FIP200, mAtg13, and Atg101 create a protein complex that can be influenced by post-translational alterations, such as phosphorylation and acetylation. ULK1's transcriptional and posttranscriptional alterations differ in various malignancies, making it a prospective therapeutic target as well as a diagnostic marker. ATG1 is the first autophagy-related gene (ATG) discovered in yeast. It is a phosphorylation-dependent regulation mechanism that includes Atg1 and seven additional interacting proteins.[68] The non-coordinated-51 (Unc-51) nematode homolog Atg1 plays a similar and extra neuronal role. Vertebrate genomes contain five kinases that are closely related to Atg1, but only Unc-51-like protein kinase 1 is present. ULK1 and ULK2 have autophagic capabilities as well as a new vesicular transport mechanism that is specific to neurons.[69] The molecular regulation of autophagy is facilitated by the ULK1 complex pre-initiation, the phosphatidylinositol 3-kinase complex initiation class III, and two protein-like (Ub-like) protein conjunction systems. ULK1 forms preinitiation complex, mAtg13, focal adhesion kinase (FAK) 200 KDa (FIP200), and Atg101. Family interacting protein and can be generated by

Table 1: Regulation of autophagy through proteins and genes

		Regulation of autophagy
Sr. No.	**Protein / Genes involved in autophagy**	**Role of proteins and genes**
1	FIP200	A component of the ULK1/2 complex that governs autophagosome formation induction in the early stages.[40]
2	5 AMPK	An enzyme that is essential for regulating ATP generation and maintaining cell homeostasis.[41]
3	mTOR (Mammalian target of rapamycin)	Autophagy is activated during ageing and is connected with negative feedback in the rapamycin (mTOR) pathway PI3K - mammalian target. PIKK family serine/threonine-protein kinase that regulates several cellular functions such as metabolism, cell proliferation, and autophagy.[42]
4	ULK1/2 (Unc-51-like autophagy activating kinase $\frac{1}{2}$)	Two ATG1 serine/threonine kinase homologues that are required for autophagy signalling. ULK1 phosphorylates mAtg13 and FIP200 specifically, indicating unambiguous roles in autophagy for the most reliable evidence of Atg1 substrates to date.[43,44]
5	VPS34 (Vacuolar protein sorting protein 34)	Class III PI3 K protein that collaborates with phosphorylating phosphatidylinositol to create phosphatidylinositol (3)-phosphate (PI3P), which is required for autophagosome formation. In the wild, Purification of the ubiquitin proteome that accumulated in VPS34-inhibited cells led to the discovery of various autophagic substrates, including NCOA4, which physically binds to the ferritin protein complex and leads it to autolysosomes for destruction.[45,46]
6	ATG7 (Autophagy-related protein 7)	E1, a homologous ubiquitin-activating enzyme, attaches to and activates LC3 in preparation for conjugation.[47]
7	ATG13 (Autophagy-related protein 13)	The autophagy protein targets the mTOR signalling system, which regulates autophagosome production via phosphorylation.[48]
8	ATG3 (Autophagy-related protein 3)	Following ATG7 activation by LC3, a homologous ubiquitin-conjugating enzyme E2 mediates the conjugation of LC3-I to phosphatidylethanolamine.[49]
9	ATG4B (Autophagy-related protein 4B)	A cysteine peptidase enzyme identical to ATG4 that cleaves the terminal residues of proLC3, allowing phospholipid conjugation and promoting autophagosome formation.[40]
10	LC3 Microtubule-associated proteins 1A/1 B light chain 3B (LC3)	Ubiquitin-like protein (LC3-I) that combines LC3-II with phospholipid phosphatidyl-ethanolamine and is involved in autophagosome formation.[50]
11	PI3K (Phosphoinositide 3-kinase)	A signal transducer enzyme class inside cells that regulates cellular function through phosphorylation of the lipids associated with a phosphatidylinositol (PI).[16]
12	P62	Protein kinase C-interacting protein is used to signal and activate the mTOR pathway; destroyed by autophagy. P62 itself is a scaffold protein binding essential signaling intermediaries across various domains.[51,52]
13	BECL1	A major PI3 K protein that interacts with BCL-2 or Pl3 K and plays a crucial role in activating and controlling autophagy and apoptosis.[53]

Table 2: Role of autophagy proteins in cancer

Cancer type	**Protein**	**Phase of autophagy**	**Status in cancer**
Colorectal carcinomas	AMBRA1	Initiation	Mutated[54]
Breast carcinomas	FIP200	Initiation	Mutated[55]
Breast carcinomas	BECN1	Initiation	Decreased[56]
Colorectal and gastric carcinomas	BECN1	Initiation	Increased.[57]
Melanoma	ATG5	Elongation	Decreased.[58]
Benign liver tumour	ATG5	Elongation	Decreased[59]
Colorectal and gastric carcinomas	ATG5	Elongation	Mutated[60]
Fibrosarcomas	ATG4C	Elongation	Decreased[58]
Leukaemia	RAB7A	Fusion	Mutated[61]
Hepatocellular carcinomas	ULK1	Initiation	Increased[62]
Breast carcinomas	ULK1	Initiation	Increased[63]
Oesophagal squamous cell carcinomas	ULK1	Initiation	Increased[35]

stress signals. Such as amino acid or serum malnutrition, low energy, and lack of glucose. Notably, Atg13 and FIP200 are essential for autophagosome localization from ULK1 to the isolation membrane. Homeostatic regulators such as mammalian TOR complex 1 (mTORC1) and AMP-activated kinase (AMPK) activate ULK1 through post-translation alterations.[70] MTOR is a well-known regulator of the ULK1 complex; it phosphorylated ULK1 directly, rendering it inactive. Also, mTORC1 regulates trafficking in Atg9, which is the only autophagic multiple membrane proteins that spans[43] as an energy device AMPK can makeULK1 activity by inhibiting mTORC1 and phosphorylating ULK1 directly under deprivation condition.[71]

The Structure of ULK1

Atg1, which was discovered by genetic screening in *Saccharomyces cerevisiae*, plays energetic downstream role for the nutrition sensor mTOR. This has a rare binding partner including Atg13, Atg15, Atg17, Atg20, Atg24, Atg29, Atg31, and others. Among these proteins, Atg1 and Atg13 play roles in autophagy as well as autophagy-related targeting (Cvt) between the cytoplasm and the vacuole. In contrast, Atg17, Atg29, and Atg31 are directly involved in autophagy, whereas Atg11, Atg20, and Atg24 are only involved in Cvt pathways.[72] Only ULK1/2/3/4 and STK36 are Atg1 orthologs prearranged by vertebrate genomes, and only ULK1 and ULK2 are available for autophagy control. Human ULK1 has a 41% total similarity to Unc-51, a *Caenorhabditis elegans* homolog, and a 29% similarity to Atg1. Similar to the other related kinases ULK3, 4, and STK36 where similarity is restricted to the catalytic N-terminal domain, the similarity among ULK1 and Atg1 includes the complete protein, including the catalytic N-terminal domain(NTD), the central proline / serine-rich (PS) domain, and the C-terminal domain (CTD).[73] The N-terminal kinase of the ULK1 domain (residues 16-278) and the CTD (residues 833-1050) are largely conserved in Homo sapiens. A PS region containing post-translational modification sites is found between the kinase domain and the CTD. ULK2 has a combined association of 52% of amino acids with ULK1; it is therefore accused of compensating for ULK1 functions or playing its roles in initiating autophagy.[74] Furthermore, ULK1 knockdown or dominant-negative mutations may cause autophagy to be disrupted, showing that ULK1 is an important autophagic protein kinase. CTD, as a highly conserved area, may also fulfil other important activities. Changes in autophosphorylation and conformation in the kinase-dead mutants with CTD treatment, as well as maps of areas directly affecting membrane connection and interaction between ULK1 and mAtg13, established the dominant-negative behavior of the a7-residue motif within CTD. This implies that CTD's research may not be limited to interacting with mAtg13, but may possibly interact with other functions.[68]

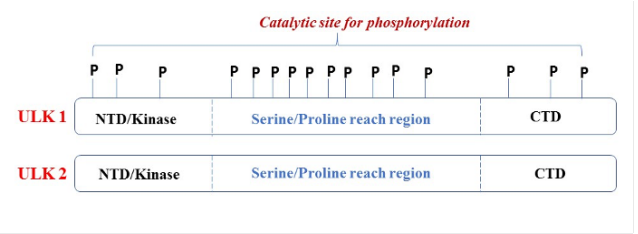


Fig. 2: Structure of ULK1 andULK2 (NTD/Kinase- N Terminal kinase domain, CTD-C Terminal Kinase domain)

Amino acid recycling via autophagy

As a result of amino acid deficiency, autophagy is strongly stimulated, as well as lysosomal destruction of cell proteins, thereby restoring amino acid levels as autophagic leucine is reprocessed at the lysosome by SLC38A9, cytosolic leucine levels are replenished and mTOR is activated.[75] This allows mTOR amino acids shaped by autophagy to repress autophagy, resulting in a negative response loop among. autophagy and mTOR activity.[76] By increasing principal amino acid transporters (AATs) such as SLC6A9, SLC7A1, SLC7A5, and others when autophagy is impaired.[77] ATF4 transcriptional targets increase in response to abstaining cues, especially when self-eating process is blocked. Tumour cells with autophagy deficiencies died when glutamine deprivation was applied. Around 80% of all cellular ribonucleotides and approximately half of all cellular amino acids are found in ribosomes, including arginine, lysine, and histidine. Ribophagy is started by hunger, and ribosomes are turned over to provide. amino acids and nucleosides.[24] When cells are hungry, NUFIP1 translocate to autophagosomes and communicates with LC3 and small. nucleolar ribonucleoproteins. NUFIP1 has an LIR motif that promotes its. interaction with LC3 and is required to deliver lysosomal ribosomes for degradation.[75]

Upstream regulation of the ULK1/2 & Atg13-FIP200 complex

Stress-related pathways are incorporated by the three majors signaling nodes mTORC1, AMPK, and p53 and transmitted to Ulk1/2-Atg13-FIP200. The Ulk1/2-Atg13-FIP200 complex is controlled by mTORC1. The signaling of the growth factor positively regulates the catalytic activity of mTORC1 itself via the class I PI3KAkt pathway, either by TSC1/2 inhibition.[78–80] Initiation of autophagy can be controlled positively by AMP-activated protein kinase (AMPK), which is activated by ATP/AMP ratios.[81,82] This is accomplished by inhibiting mTORC1 through TSC1/2[83] or by direct phosphorylation of the raptor portion mTORC1.[84] Recent studies have shown that AMPK can also activate Ulk1 and Ulk2, directly regulating Ulk1/2 kinase.[85–87] While, mTORC1 negatively controls the interaction among AMPK and Ulk1/2 (13). Hypoxia, DNA damage, and

Table 3: Phosphorylation sites on components of the ULK1 complex

Protein	Residue	Kinase	Location in structure	Functional effect
ULK1	T180	ULK1 auto phosphorylation	Activation loop in the catalytic domain	Stimulates ULK1 kinase activity [100]
	S317	AMPK	IDR	Stimulates ULK1 kinase activity [13]
	S467	AMPK	IDR	Required for mitochondrial homeostasis during starvation. [86]
	S555	AMPK	IDR	Required for mitochondrial homeostasis during starvation, regulates Atg9 localization [86,101,102]
	T574	AMPK	IDR	Required for mitochondrial homeostasis during starvation [86]
	S637	mTORC1 AMPK	IDR	Encourages ULK1-AMPK interaction During starvation, it is required for mitochondrial homeostasis and affects Atg9 localization. [101]
	S757	mTORC1	IDR	Inhibits ULK1 kinase activity [86]
	S777	AMPK	IDR	near N-terminus of EAT domain [86]
ATG13	S224	AMPK	IDR	Inhibits ULK1 kinase activity [99]
	S258	mTORC1	IDR	Inhibits ULK1 kinase activity [99]

oncogenic stress activate the tumour suppressor protein p53. It is important to remember that p53 is both a negative and positive autophagy controller.[88] Activated p53 induces autophagy via AMPK-TSC1/2 inhibition of mTORC1 activation,[89] most likely by transcriptionally up-regulating AMPKb-1/2, TSC2[90] and Sestrin1/2[91,92] or by up-regulating other pro-autophagic factors, such as the damage-regulated autophagy modulator.[93] As well as Ulk1, Ulk2 have been identified as p53 transcription targets[94] and cytoplasmic p53 has been discovered to negatively regulate autophagy in a hitherto unknown way.[95,96] Nonetheless, this cytoplasmic property appears to be intimately related to its capacity to directly interact with FIP200, as a single mutation in p53 (K382R) removes both the FIP200 binding and the anti-autophagic capability.[88] This schizophrenic activity of p53 in the regulation of autophagy may seem confusing at first glance. However, in terms of cell survival, the double-edged existence of p53 has already been known, Welles. Low basal p53 levels are pro-survival under normal development conditions, while high p53 levels have the opposite effect under extreme stress conditions. It has been proposed that a low level of p53 activity is also anti-autophagic (Especially when p53 deficiency causes autophagy even under normal development circumstances). Only active p53, on the other hand, is pro-autophagic, especially under situations of cellular duress such as oncogenic or genotoxic stress.[88] ULK1 is influenced by amino acid and energy status via the mTORC1 (mechanical target rapamycin-1) and AMPK kinases (AMP-activated protein kinase). mTORC1 stimulates protein synthesis and other anabolic processes intricate in cell development and digestion by combining growth hormones, oxygen levels, amino acids, and energy status.[97] When mTORC1 is activated, it reduces the movement of ULK1 and ATG13, which inhibits autophagy.[44] Table 5 describes the phosphorylation sites. ULK1 inhibition prevents rapamycin from activating autophagy, demonstrating that ULK1 regulation is a critical step in the induction of autophagy downstream of mTORC1 inhibition.[98] When mTORC1 is inhibited under malnourishment, it dissociates itself from ULK1. AMPK, which assesses cellular energy status and is activated by increasing intracellular AMP, also controls ULK1.When mTORC1 is inactive than AMPK indirectly activates the ULK1 complex, and it is also directly phosphorylates ULK1 at several sites in the interconnecting region between the kinase and C-terminal domains, and this has a starting point of autophagy, although one study showed an inhibitory effect[86,99] AMPK also phosphorylates ATG13.[99]

Ulk1 promote tumour growth

Autophagy is widely known in the biological cycle for its dual role duties of cyto- protection and cytotoxicity. Cytoprotective autophagy characteristics benefit cancer cells in a variability of circumstances during cancer production and progression, such as genomic instability, survival under nutritional deprivation, hypoxia, high ROS, and so on. Because the ULK1 complex is an autophagic originator, cytoprotective autophagy may be a promising target for reducing cancer progression, much interest has been intensive on understanding its character in cancer formation. ULK1 may promote the viability of cancer cells by allowing the survival of cancer cells under hypoxia, a significant feature of tumor cells in vivo that has led to the discrepancy between high proliferative levels and the lack of nutritional supply in the blood. Also, severe hypoxia can stress the endoplasmic reticulum (ER). ULK1 also participates in the

integrated stress response to protect cells from ER stress, and the ablation of ULK1 results in caspase-3/7-independent death of cells.[63]

ULK1/2 suppresses tumor growth

Autophagy is a homeostatic system that, when interrupted, can encourage and accelerate cancer. Autophagy suppresses cancers by eliminating damaged organelles/proteins and decreasing cell explosion and genetic instability.[103] Cytotoxic autophagy or autophagic cell death, in accumulation to cytoprotective autophagy, is common in malignancies. Autophagy controllers, such as Beclin-1, are tumour suppressor-positive.[104] Beclin 1 is an autophagy-inducing protein that is tumour resistant to Beclin 1/mice, implying that Beclin 1 is a tumour suppressor gene that is insufficient.[105] A possible molecular link among flawed autophagy and tumorigenesis includes the growth of p62/SQSTM 1 protein aggregates, impaired mitochondria, and defective proteins leading to reactive oxygen species (ROS) growth. It causes damage to DNA, which may lead to genomic instability. Self-eating process can also protect against tumorigenesis by reducing necrosis and chronic inflammation associated with HMGB1 release.[106] There are also a few medicines that target anticancer qualities by promoting cell autophagy. For example, Rapamycin inhibits mTORC1, and Beclin-1 expression increases with Tamoxifen, a well-known breast cancer drug.[107]

ULK1 Mediates Signaling Cancer Therapy pathways

Autophagy is usually used as a tool for shielding human cells from harmful effects conditions.[111] Several trials Suggested that the purpose of autophagy can differ over the tumor development stages.[112,113] Autophagy was also originating to influence tumor growth in cell- or tissue-specific ways. So, autophagy plays a role double function in tumor growth.[114] Autophagy is when opposed to other cell functions relatively drug-able and selective. At the moment just a few enzymes are targeted during autophagy drugs, even though we learn more than 36 proteins connected to autophagy.[115] ULK1 begin the autophagy process on. So that may be a viable target for narcotics. As a sponsor of autophagy, ULK1 often plays different functions depending on the tumor forms and levels. For example, low ULK1 expression in operable breast cancer tissues has been revealed to be a predictor of poor prognosis.[116] It is significant to highlight that tumors do not form in segregation but are surrounded by nearby tissues that may be harmed by treatment outcomes. In these circumstances, the connection of autophagy in both tumor cells and surrounding cells must be considered. For example, while autophagy inhibition has sensitized MDA-MB-231 (but not MCF-7) cells to radiotherapy, autophagy is essential to avoid the effect of senescent tumor cells on non-irradiated cells on by standers.[117] While inducing senescence may be advantageous in slowing tumor growth, soluble substances released by senescent cells are thought to be harmful.[118] Another important consideration when examining the impact of autophagy on the success of anticancer therapy is the involvement of autophagy in the immunological response to anti-tumor therapy. Some of the most significant roles of autophagy, which must be thoroughly explored and researched, are based on in-vivo experiments and clinical results. At the moment, anti-tumor immune response is a fast-expanding area of cancer therapy. While conventional therapy may slow disease development, it frequently fails to achieve complete remission or prevent recurrence, and the immune response is thought to be dangerous for tumor cell clearance. Whether or not autophagy plays important role in cancer immunity, its role in encouraging an immune response may be as essential as its role as a key response to anti-tumor action.

Inhibition of ULK1-Mediated Autophagy for Cancer Treatment

Autophagy supplies tumor cells with the building blocks and resources in response to oxidative pressure and chemotherapeutic drug toxicity, encouraging tumor cell existence and growth.[135] A rising number of researchers have discovered that autophagy inhibition is potential e-mechanism for tumor therapy.[136,137] This dynamic mechanism requires proteins. One way of preventing the protein degradation process can be targeted at these proteins.[138,139] The crystal structure was determined in ULK1,2.[108]Table 4 shows the various Ulk1/2 inhibitors which inhibit cancer cells in the different cell lines. Compounds identified by Petherick et al., MRT67307 and MRT68921, which were successful in inhibiting ULK1 (45.0 and 2.9 nM IC50 levels, The IC50 (IC50 values of 38.0 and 1.1 nM, respectively) and the ULK2 expression in the in vitro kinase Assay and avoidance of autophagy in embryonic fibroblast cells (MEFs) in the mouse.[98] In Studies, It was observed that the compound prevents the autophagy of a drug-resistant ULK1 mutant cell line Tumor cells have strong inhibitions of ULK1 activation.[98] To improve ULK1's selectivity the novel ULK1 scaffold inhibitor was investigated, significant to the detection of other compounds. Potent ULK1 and ULK2 antagonists with strong selectivity.[108] SBI-0206965, an FAK regulator, has been revealed to be a highly selective ULK1 and ULK2 inhibitor that operates by decreasing ULK1-averaged Vps34 phosphorylation in tumour cells[140,141] SBI-0206965: In surgical rehabilitation synergizes to destroy cancer cells through the mTOR inhibitors.[142]

CONCLUSION AND PERSPECTIVE

The ULK1 complex have significant role in the initiation of autophagy in response to various stressors; it is tightly regulated via post-translational modification and protein-

Table 4: Some Selected ULK1/2 containing Compounds That Modulate Autophagy

Compound	Chemical structure *	Target	Novel features	Potency / Selectivity
Compound 1	A	ULK1 and ULK2	Inhibitor Pyrazole amino quinazoline scaffold Crystal structure obtained with ULK1	ULK1: IC50 of 5.3 nM; [108] ULK2: IC50 of 13 nM; [108] PDPK1: IC50 of 420 nM [108]
Compound 3	B	ULK1 Inhibitor	Pyrimidine scaffold Crystal structure obtained with ULK1	ULK1: IC50 of 120 nM; [108] ULK2: IC50 of 360 nM; [108] PDPK1: IC50 of 710 nM [108]
SBI-0206965	C	ULK1 and ULK2	Selective inhibitor Pyrimidine scaffold Suppresses ULK1 downstream phosphorylation of VPS34 and Beclin-1 Induces apoptosis in NSCLC cells by destabilizing Bcl2 and Bclxl	ULK1: IC50 of 108 nM; [109] ULK2: IC50 of 711 nM [109]
MRT67307	D	ULK1 and ULK2	In vitro inhibitor Pyrimidine scaffold Also targets TBK1 and AMPK-related kinases	ULK1: IC50 of 45 nM; [98] ULK2: IC50 of 38 nM [98]
MRT68921	E	ULK1 and ULK2	In vitro inhibitor Pyrimidine scaffold Also targets TBK1 and AMPK-related kinases	ULK1: IC50 of 2.9 nM; [98] ULK2: IC50 of 1.1 nM [98]
SR-17398	F	ULK1	Indazole-derived inhibitor Mixture of four stereoisomers	ULK1: IC50 of 22 mM [110]
SR-20295	G	ULK1	Indazole-derived inhibitor	ULK1: IC50 of 45 nM, In vitro microsome [110]

* Chemical structure placed as sperate Figure 3

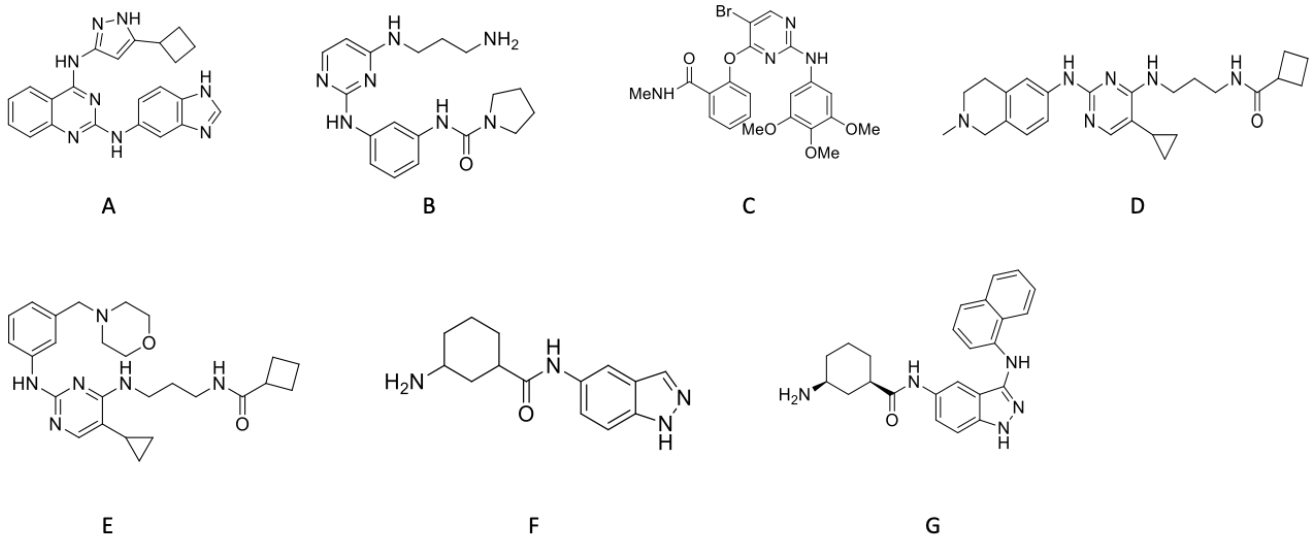


Fig. 3: Chemical structure in reference to Table 4

protein interactions. Homologs of Atg1, ULK1, and ULK2 were discovered in the 1990s, with research on ULK1 rapidly expanding. Recent review has finding a variety of mechanisms in ULK1-regulated autophagy, including the existence of AMPK- or mTORC1-independent independent initiation of the ULK1 complex, as well as the activity of the novel-related protein Atg101. ULK1 has been identified as a novel target for cancer diagnosis because of its diverse expression and biological role in cancer cells. Diverse pathways involved in autophagy activation and autophagosome formation have provided researchers with numerous options for targeting ULK1 or its related network in cancer therapy.

Table 5: Summary of breast cancer treatments inducing autophagy

Cell line	Drug	Autophagy Role	Autophagy Proteins
LCC-1, LCC-9	Fulvestrant	Protective	P62/SQSTM1 degradation, LC3I/LC3II conversion, LC3-GFP puncta [119]
SK-ER, MCF-7	ICI-182780	Protective	LC3I/LC3II conversion, LC3-GFP-RFP puncta, ATG-7 upregulation [120]
MCF-7 T47D	Tamoxifen	Protective	LC3GFP puncta [121]
MCF-7	Tamoxifen, raloxifene	Protective	LC3I/LC3II conversion, [122] LC3-GFP puncta [122]
MCF-7	Exemestane, Synthetic Aromatase Inhibitors 5α-androst-3-en-17-one and 3α,4α-epoxy-5α-androstan-17-one	Protective	Not shown [123]
MCF-7	Exemestane	Protective	LC3I/LC3II conversion [124]
SKBR3, BT474, MCF7, JIMT-1	Gefitinib	Protective	LC3I/LC3II conversion, p62/SQSTM1 degradation, LC3-GFP puncta [125]
SKBR3, JIMT-1	Trastuzumab	Protective	LC3I/LC3II conversion, p62 degradation, LC3- GFP puncta [126]
JIMT1, SKBR3	Trastuzumab Lapatinib	Protective	LC3I/LC3II conversion, p62 degradation, LC3- GFP puncta [127]
MDA-MB-231, MDA-MB-453	Cisplatin Docetaxel	Cytotoxic Protective	GABARAPL1, GABARAPL2, HDAC6, IRGM, MAP1LC3B and ULK1 upregulation, LC3I/LC3II conversion,p62 degradation [128]
MCF-7, ZR-75, T47D	Palbociclib	Nonprotective. Autophagy ablation increases drug sensitivity through onset ofsenescence	LC3I/LC3II conversion, p62/SQSTM1 degradation, LC3-GFP puncta [129]
MCF-7, T47D MDA-MB-231	Camptothecin	Protective & Nonprotective	LC3-GFP puncta, LC3II/Lysosomal Marker LAMP1 colocalization. [130]
MDA-MB-231, MDA-MB-436, BT-549	Adriamycin, Cyclophosphamide	Protective	LC3I/LC3II conversion, RFPGFP- LC3 puncta [131]
MCF-7	Adriamycin	Cytotoxic	LC3I/LC3II conversion, Beclin1 upregulation [132]
Tamoxifen resistant MCF-7	SAHA (Suberoylanilidehydroxamic acid)	Cytotoxic	LC3I/LC3II conversion, [133] Atg5, Atg7 upregulation [133]
4T1	Histone Deacetylase Inhibitor TMU- 35435 (in combination with Etoposide)	Cytotoxic	LC3I/LC3II conversion, [134]

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