Autophagy and beyond: Unraveling the complexity of UNC-51-like kinase 1 (ULK1) from biological functions to therapeutic implications

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\textbf{Abstract} UNC-51-like kinase 1 (ULK1), as a serine/threonine kinase, is an autophagic initiator in mammals and a homologous protein of autophagy related protein (Atg) 1 in yeast and of UNC-51 in Caenorhabditis elegans. ULK1 is well-known for autophagy activation, which is evolutionarily conserved in protein transport and indispensable to maintain cell homeostasis. As the direct target of energy and nutrition-sensing kinase, ULK1 may contribute to the distribution and utilization of cellular resources in response to metabolism and is closely associated with multiple pathophysiological processes. Moreover, ULK1 has been widely reported to play a crucial role in human diseases, including cancer, neurodegenerative diseases, cardiovascular disease, and infections, and subsequently targeted small-molecule inhibitors or activators are also demonstrated. Interestingly, the non-autophagy function of ULK1 has been emerging, indicating that non-autophagy-relevant ULK1 signaling network is also linked with diseases under some specific contexts. Therefore, in this review, we summarized the structure and functions of ULK1 as an autophagic initiator, with a focus on some new approaches, and further elucidated the key roles of ULK1 in autophagy and non-autophagy. Additionally, we also discussed the relationships between ULK1 and human diseases, as well as illustrated a rapid progress for better understanding of the discovery of more candidate small-molecule drugs targeting ULK1, which will provide a clue on novel ULK1-targeted therapeutics in the future.
1. Introduction: A brief overview of UNC-51-like kinase 1 (ULK1)

Autophagy was proposed by Ashford and Porter after discovering the phenomenon of “eating yourself” in cells in 1962. It is an intracellular degradation system through which cytoplasmic material is transported to the lysosome and degraded, and converted into nutrient recycling for the cell. During phases of insufficient nutrient availability, autophagy will be quickly upregulated to provide energy and maintain basic cell functions. Therefore, cells starved of glucose or amino acid supply or under hypoxia rely on autophagy to preserve their survival. In general, autophagy is a kind of mechanism that promotes cellular survival by keeping energy homeostasis and removing toxic proteins, pathogens, and aged or harmful organelles. This mechanism was discovered by Yoshinori Ohsumi in a mutant Apg1 yeast in 1993, and for this, he won the 2016 Nobel Prize in Physiology or Medicine. Intriguingly, Apg1, also known as Atg1, was the first protein found to be involved in the initiation of autophagy in yeast. The following year, human homologous protein ULK1 (UNC-51) was isolated by Ogura and his colleagues from mutants of C. elegans displaying aberrant axon extension and development, which is encoding a new Ser/Thr kinase. Since then, pioneering creations about autophagy initiator Atg1/ULK1 were continuously made by numerous labs around the world. In special, the molecular and biochemical approaches revealed miscellaneous binding partners, upstream regulators/downstream effectors, signaling mediators of Atg1/ULK1, which bring holds promise for the treatment of autophagy-related diseases. For example, in yeast, Atg1 is complexed with Atg13 and Atg17, which are necessary for autophagosome formation. Similarly, in mammals, ULK1 also interacts with mATG13, ATG101, and FIP200 binding proteins to activate autophagy in the form of complex es. In addition, it was found that ULK1 is not only a downstream effector of mammalian target of rapamycin (mTOR) but also phosphorylated and activated by AMP-activated protein kinase (AMPK). Due to the evolutionary conservation of Atg1/ULK1 in protein trafficking, energy- and nutrient-sensing kinases can directly regulate Atg1/ULK1 to regulate the distribution and utilization of cellular resources to maintain cellular homeostasis in changing complex physiological environments in vivo. Therefore, ULK1 is necessary for the occurrence and development of diseases, associated with autophagy or non-autophagy pathways. Certainly, ULK1 has been proved to regulate cancer, neurodegenerative disorders, infection, cardiopathy, and other diseases. Further, a sequence of small molecule compounds targeting ULK1 has been revealed good therapeutic efficacy for multiple human diseases. Shaw’s lab discovered and synthesized ULK1 kinase inhibitor SBI-0206965 with high selectivity, which inhibited ULK1-mediated phosphorylation to regulate autophagy and cell survival. In 2017, first ULK1 targeted activator LYN-1604 was designed and discovered by Liu’s lab based on computer-aided drug design methods and screened with kinase and antiproliferative activity. Importantly, LYN-1604 exhibited good therapeutic potential for breast cancer by activation of ULK1 and ULK1 complex (Fig. 1).

Thus, in this review, we focused on summarizing the molecular structures and biological functions of ULK1 in autophagy and beyond autophagy. Besides, we clarified the complex and elaborate protein–protein interaction (PPI) network in which ULK1 resides, its direct regulators, indirect regulators, and substrates. In addition, we introduced the key signaling pathways and molecular mechanism which contributed to complete the role of ULK1 in different human diseases, associated with its autophagy and non-autophagy functions. Moreover, we further discussed the prospects of drug development targeting ULK1 and novel ULK1- for the future therapeutic applications.

2. Molecular structures of ULK1 and its complex

2.1. The structure of ULK1

ULK1 protein composed of 1051 amino acids has a molecular weight of 112 kDa and consists of an N-terminal kinase domain (KD); a C-terminal domain (CTD) for ULK1 and its complex to interact with; as well as a proline/serine (PS) for the most post-translational modification. For ULK1, KD promotes kinase activity, while CTD contains two microtubule-interacting and transport (MIT) domains that encourage the interaction of ULK1 with mATG13 and FIP200. The PS region connecting KD and MIT are not well conserved, formed from about 500 amino acids, which is mainly responsible for the interaction of ULK1 with autophagic microtubule-associated protein light chain 3 (LC3) and the phosphorylation modification of ULK1 by upstream kinases (AMPK, etc.). Notably, KD and MIT exhibit high sequence conservation in the Atg1/ULK1 family of kinases, both in yeast and mammals, in comparison to the previously mentioned regions of the future therapeutic applications.
ULK1 typically functions by forming a tetrameric complex with ATG13, ATG101, and FIP200 to initiate starvation-induced autophagy. A corresponding analogy is also in yeast, where Atg1 becomes a pentameric complex with Atg13 (the ULK1 and ATG13 homolog), Atg17 (the mammalian FIP200 homolog), Atg29, and Atg31, which are the most upstream autophagy initiation factor. After forming a complex with Atg29 and Atg31, Atg17 further interacts with Atg1 and Atg13 to promote the organization of pre-autophagosome structures upon starvation. In addition, in C. elegans, EPG-9 (homologous gene of ATG101) directly interacts with EPG-1 (homologous gene of mAtg13/Atg13) and EPG-7 and forms a complex in the aggrephagy pathway to initiate autophagy.

ULK1 regulates the initiation of autophagy by forming the ULK1–ATG13–FIP200–ATG101 stable complex. Therein, ATG13 and FIP200 cooperate in the autophagy process. ATG13 has a very short peptide motif at the C-terminus that connects to ULK1, and it also acts as an adaptor protein to recruit components of the ULK1 complex, including ULK1, FIP200, and ATG101, to the core to initiate autophagy. As another component of the ULK1 complex, ATG101 is a stabilizer of ATG13 and an auxiliary protein of the complex, which is not regulated by nutritional conditions. It also functions as a bridge to link ULK1 and the downstream PtdIns3K complex during mammalian autophagy.

ULK1 is regulated by transcriptional and post-transcriptional mechanisms (Table 1). Atg17–Atg31–Atg29 complex is a double dimer and migrates to pre-autophagosome, with Atg31 acting as the bridge between the other two proteins. This curved structure helps the complex bind Atg1–Atg13 to ATG-containing vesicles, which may be the source of the membrane formed by the autophagosome.

The situation in C. elegans is relatively simple. The EPG-9–EPG-1 complex accumulates in C. elegans and then interacts with UNC-51. Among them, EPG-1 is similar to Atg13 in yeast. EPG-9 could form a complex with EPG-1 by direct interaction in autophagy process in C. elegans. EPG-7, a protein similar to human FIP200 and yeast Atg11, acts as a scaffold protein but is unnecessary for starvation-induced autophagic degradation. In addition, in C. elegans, UNC-51 directly binds and affects the activities of VAB-8 and UNC-14, where VAB-8 is involved in axonal backward migration and growth, and UNC-14 regulates UNC-5-related sub-cellular localization and actin 1-dependent synaptic vesicle transport. These two proteins are involved in axon conduction and growth and are independent compared to autophagic proteins EPG-1 and EPG-9.
transcription of ULK1 and other Atg genes to initiate autophagy \[^44,45\]. In nonalcoholic hepatic steatosis, Hnf4a could directly activate the transcription of ULK1 to stimulate autophagy \[^46\]. ATF4, a known endoplasmic reticulum (ER) stress regulator, could transcriptional up-regulation of ULK1 in response to severe cellular stress, including hypoxia, ER stress and anti-angiogenic therapy \[^47\]. STAT1 is a transcriptional suppressor of ULK1, which negatively regulates the mRNA and protein levels of ULK1 \[^48\]. Likewise, the transcriptional repressor DAXX inhibits the expression of ULK1 in vivo to suppress autophagy, promoting PCa tumorigenicity \[^49\]. Additionally, cAMP response element-binding protein (CREB) is a new transcriptional activator of autophagy, which upregulates the autophagy gene ULK1 \[^50\].

Further, nuclear receptor subfamily 1 group D member 1 (NR1D1) can directly enhance the transcription of ULK1 during spermatogenesis in mice. Interestingly, STRA8 can inhibit the expression of ULK1 \[^51\]. Intriguingly, high mobility group nucleosomal binding domain 2 (HMGN2) and H3K27ac co-regulates the transcription and expression of ULK1. Of them, HMGN2 recruitment at the ULK1 promoter to accelerate ULK1 expression while H3K27ac negatively affect the transcription of ULK1 \[^52\]. Moreover, the spliceosome PRPF8 was found to facilitate mitophagy by affecting ULK1 mRNA splicing and might result in retinitis pigmentosa \[^53\]. PGC-1α, a co-regulator of gene expression in mitochondrial biogenesis, promotes autophagy and mitophagy through ULK1 to reduce NLRP3 inflammasome, suppressing neuroinflammation in acute ischemic stroke \[^54\] (Fig. 3A).

### 3.1.2. Post-transcriptional regulation

Post-transcriptional gene regulation is another key regulatory mechanism of gene expression, mainly including the process of RNA splicing, RNA interference, MicroRNAs, RNA editing, messenger RNA (mRNA) stability and others \[^55\]. As to ULK1, its post-transcriptional regulation is also diverse. The current research on the post-transcriptional regulation of ULK1 mainly focuses on the factors that regulate ULK1 mRNA, including RNA helicase, microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs). Recently, researchers found that
| No. | Name               | Classification         | Positive regulation/ negative regulation | Mechanism                                                                 | Ref. |
|-----|--------------------|------------------------|------------------------------------------|---------------------------------------------------------------------------|------|
| 1   | ZFP36L2            | Gene                   | Positive regulation                      | ZFP36L2 positively regulates the transcription level and protein expression level of ULK1 | 41   |
| 2   | RAB13              | Gene                   | Positive regulation                      | RAB13 positively regulates the transcription level and protein expression level of ULK1 | 41   |
| 3   | NFE2L2/NRF2        | Transcription factors  | Positive regulation                      | NFE2L2/NRF2 activates the expression of ULK1                              | 42   |
| 4   | NF-E2              | Transcription factors  | Positive regulation                      | NF-E2 increase the expression of ULK1                                    | 43   |
| 5   | FOXO1              | Transcription factors  | Positive regulation                      | FoxO1 promotes the expression of ULK1                                     | 44   |
| 6   | FOXO3              | Transcription factors  | Positive regulation                      | FoxO3 activation increases the abundance of mRNA and protein levels of ULK1 | 45   |
| 7   | Hnf4α              | Transcription factors  | Positive regulation                      | Hnf4α directly activating transcription of ULK1                           | 46   |
| 8   | miR-214-3p         | MicroRNA               | Negative regulation                      | Suppressing ULK1 expression through direct binding at a 3’ untranslated region sequence | 46   |
| 9   | ATF4               | Transcription factors  | Positive regulation                      | Driving ULK1 mRNA and protein expression in severe hypoxia and ER stress  | 47   |
| 10  | STAT1              | Transcription factors  | Negative regulation                      | STAT1 bound a putative regulatory sequence in the ULK1 5’-flanking region, the mutation of which increased ULK1 activator activity | 48   |
| 11  | DAXX               | Transcription factors  | Negative regulation                      | Repressing expression of Ulk1 in vivo                                     | 49   |
| 12  | CREB               | Transcription factors  | Positive regulation                      | Up-regulation of autophagy genes, including Arg7, ULK1, and Tfeb, by recruiting the coactivator CRTC2 | 50   |
| 13  | Nr1d1              | Protein                | Positive regulation                      | Nr1d1 activates ULK1 expression by engaging directly on the distal ROREs to enhance its transcription. | 51   |
| 14  | STRA8              | Protein                | Negative regulation                      | STRA8 binds to the Nr1d1 promoter to inhibit Nr1d1 expression thus to inhibit Nr1d1-regulated ULK1 up-regulation | 51   |
| 15  | p53                | Protein                | Positive regulation                      | Phospho-p53 Ser392 increases Ulk1 expression by binding to ULK1 promoter   | 52   |
| 16  | HMGAI              | Protein                | Negative regulation                      | HMGAI1 binds ULK1 promoter region and negatively regulates its transcription | 53   |
| 17  | HMGN2              | Protein                | Positive regulation                      | HMGN2 promotes the transcription of ULK1                                  | 54   |
| 18  | H3K27ac            | Protein                | Negative regulation                      | H3K27ac inhibits the transcription of ULK1                                | 54   |
| 19  | PRPF8              | Spliceosome            | Positive regulation                      | Regulating Ulk1 mRNA splicing                                            | 55   |
| 20  | PGC-1α             | Transcription co-activators | Positive regulation                  | PGC-1α may regulate ULK1 expression in coordination with ERRα             | 56   |
| 21  | DDX3               | ATP-dependent RNA helicase | Positive regulation              | DDX3 promotes ULK1 expression post-translationally, which may act by binding to ULK1 mRNA | 58   |
| 22  | miR-372            | MicroRNA               | Negative regulation                      | Decreasing the ULK1 expression by binding sequences in the 3’ UTR of ULK1 | 61   |
| 23  | miR-93             | MicroRNA               | Negative regulation                      | Reducing the protein levels of ULK1 under hypoxia condition               | 62   |

(continued on next page)
| No. | Name             | Classification     | Positive regulation/negative regulation | Mechanism                                                                 | Ref.  |
|-----|------------------|--------------------|------------------------------------------|----------------------------------------------------------------------------|-------|
| 24  | miR-142-5p       | MicroRNA           | Negative regulation                      | Inhibiting translation of ULK1 mRNA though binding to the ULK1 3'UTR       | 63,102|
| 25  | miR-20a          | MicroRNA           | Negative regulation                      | Decreasing the endogenous ULK1 protein levels by binding sequences in the 3' UTR of ULK1 | 64,65 |
| 26  | miR-106b         | MicroRNA           | Negative regulation                      | Decreasing the endogenous ULK1 protein levels by binding sequences in the 3' UTR of ULK1 | 65    |
| 27  | miR-106a         | MicroRNA           | Negative regulation                      | Down-regulating ULK1, ATG7, and ATG16L1 proteins                          | 66–68 |
| 28  | miR-26a/b        | MicroRNA           | Negative regulation                      | Suppressing the post-transcriptional ULK1 expression                     | 69    |
| 29  | miR-489          | MicroRNA           | Negative regulation                      | Diminishing the expression of ULK1 and LAPT4B                              | 70    |
| 30  | miR-1262         | MicroRNA           | Negative regulation                      | Decreasing the expression of ULK1                                          | 71    |
| 31  | miR-214          | MicroRNA           | Negative regulation                      | Suppressing ULK1 expression through direct binding at a 3' untranslated region sequence | 67    |
| 32  | miR-155          | MicroRNA           | Negative regulation                      | Down-regulating the expression of ULK1, FoxO3, atg14, atg5, atg3, lc3 and GABARAPL1 | 73    |
| 33  | miR-20a-5p       | MicroRNA           | Negative regulation                      | Down-regulating the expression of endogenous ULK1                         | 74    |
| 34  | PVT1             | Long non-coding RNA| Positive regulation                      | PVT1 positively regulates ULK1 by inhibiting miR-20a-5p                   | 74    |
| 35  | miR-558          | MicroRNA           | Negative regulation                      | Down-regulating the expression of ULK1                                   | 75    |
| 36  | MALAT1           | Long non-coding RNA| Positive regulation                      | MALAT1 positively regulates ULK1 by inhibiting miR-558                    | 75    |
| 37  | SNHG6            | Long non-coding RNA| Positive regulation                      | SNHG6 positively regulates ULK1 by inhibiting miR-26a-5p                  | 76    |
| 38  | miR-26a-5p       | MicroRNA           | Negative regulation                      | Down-regulating the expression of ULK1 through directly acting on the 3'UTR of ULK1 | 76-78 |
| 39  | miR-30b-3p       | MicroRNA           | Negative regulation                      | Down-regulating the expression of ULK1                                   | 79    |
| 40  | Gm15834          | Long non-coding RNA| Positive regulation                      | Gm15834 positively regulates ULK1 by inhibiting miR-30b-3p                | 79    |
| 41  | miR-665          | MicroRNA           | Negative regulation                      | miR-665 decelerates the expression of ULK1 by binding to its 3'-UTR        | 80    |
| 42  | miR-17           | MicroRNA           | Negative regulation                      | Down-regulating the expression of f PHLPP, ULK1, ATG7 and p62             | 81    |
| 43  | PTENP1           | Long non-coding RNA| Positive regulation                      | PTENP1 positively regulates ULK1 by inhibiting miR-17 and miR-20a         | 81    |
| 44  | miR-22           | MicroRNA           | Negative regulation                      | miR-22 directly targets ULK1 to inhibit the expression of the target gene  | 82    |
| 45  | miR-34a-5p       | MicroRNA           | Negative regulation                      | Down-regulating the protein expression of ULK1                           | 83    |
| 46  | circ_0009910     | Circular RNA       | Positive regulation                      | circ_0009910 activates ULK1-induced autophagy via inhibiting miR-34a-5p   | 83    |
| 47  | miR-1275         | MicroRNA           | Negative regulation                      | Decreasing ULK1 and ATG7 expression at RNA and protein level             | 84    |
| 48  | circCDYL         | Circular RNA       | Positive regulation                      | circCDYL activates ULK1 by inhibiting miR-1275                          | 84    |
| 49  | miR-514a-3p      | MicroRNA           | Negative regulation                      | miR-514a-3p negatively modulates ULK1 expression by direct interaction    | 85    |
| 50  | miR-132-5p       | MicroRNA           | Positive regulation                      | miR-132-5p positively regulates ULK1 mRNA and protein levels             | 86    |
the post-transcriptional regulation of Atg1 (the homolog of ULK1 in yeast) was of great importance to autophagy control. In yeast, RNA-helicase Ded1 (DDX3 in mammals) was proved to promote the translation initiation of Atg1 by \textit{atg1} mRNA binding. Correspondingly, mammal DDX3 could up-regulate ULK1 expression without affecting \textit{ULK1} mRNA level, indicating this effect was post-transcriptional regulation. Importantly, miRNAs are largely contributed to the post-transcriptional regulation of ULK1. miRNAs are a series of endogenous small non-coding RNAs, approximately 17–24 bases in length, that bind to target miRNAs to achieve gene silencing and translational repression. Uridine at the 5' end of miRNA, which can partially complement the 3' untranslated region of mRNA, leading to in translation repression of the mRNA, thus, playing a vital role in post-transcriptional regulation. Numerous studies have unveiled that multiple miRNAs, including miR-372, miR-93, miR-142-5p, miR-20a, miR-106b, miR-106a, miR-26a/b, miR-489, miR-1262, miR-214, miR-214-3p, miR-125b, and miR-155 inhibit \textit{ULK1} gene expression by directly targeting the mRNA of ULK1. In addition, microRNAs could also play an indirect role by regulating ULK1-related pathways. For example, miRNAs, including miR-135b-5p, miR-378, miR-126, miR-21, and miR-99 positively regulate ULK1-related pathways, whereas miR-3473b act as a sponge in ULK1-related pathways. Of them, miR-378 initiates autophagy via mTOR/ULK1 signaling pathway and inhibits apoptosis in a cell-autonomous manner. Importantly, miRNAs are also closely related to human disease. For instance, miR-135b-5p levels are elevated in oxaliplatin-resistant colorectal cancer. Mechanistically, miR-135b-5p elevates protective autophagy in cancer cells via activation of MUL1/ULK1 signaling pathway, leading to oxaliplatin resistance. Interestingly, this provides a potential autophagy-based therapeutic strategy for preventing drug resistance. And miR-3473b, which can indirectly negative regulation of ULK1 by inhibiting TREM2, exerting a regulatory role in autophagy in the etiology and pathogenesis of inflammation in Parkinson’s disease (PD). Notably, lncRNAs may contribute a lot to the post-transcriptional regulation of ULK1. LncRNA, as a critical regulatory factor in various cellular processes, is subject to post-transcriptional monitoring to obtain sufficient stability to maintain the cellular environment and control different physiological functions.
functions and plays a critical part in the occurrence, progress, and change of multiple human diseases\textsuperscript{95}. As to ULK1, the effect of lncRNA on ULK1 mainly achieved by regulating different miRNAs, and they all positively regulate ULK1. For instance, PVT1 inhibits miR-20a-5p and thus modulates ULK1 expression\textsuperscript{74}. MALAT1 functioned as decoys sponging miR-558 to up-regulate ULK1 levels\textsuperscript{75}. Moreover, in 5-fluorouracil (5-FU)-resistant CRC cells, SNHG6 facilitates chemoresistance by antagonizing miR-26a-5p and targeting ULK1 to promote autophagy\textsuperscript{76,77}. And in hepatic carcinoma (HCC) cells, PTENP1 could indirectly restrain autophagy by inhibiting the expression of the autophagy gene ULK1 via sponging miR-17, miR-19b, and miR-20a, inducing hepatocyte tumor growth\textsuperscript{81}. Similarly, HOTAIRM1 can antagonize miR-20a, 106b, miR-125b and positively regulates their target ULK1\textsuperscript{96}. In a cardiac hypertrophy model, overexpression of miR-30b-3p inhibited autophagy-induced cardiac hypertrophy by binding endogenous to the 3’UTR of ULK1, and lncRNA Gm15834 targeted miR-30b-3p to inhibit this process and promoted cardiomyocyte autophagy, which may be the root cause of aggravating cardiac hypertrophy\textsuperscript{79}. NEAT1 targets miR-34a and promotes the expression of ULK1 to promote autophagy to enhance the sensitivity of CRC to 5-FU\textsuperscript{97}. GAS5, an anti-tumor lncRNA, positively correlates with ULK1/2 and enhances the sensitivity of tumors to chemotherapeutics in an autophagy-independent manner\textsuperscript{98}. In addition, lncRNA can affect autophagy and other physiological functions by acting indirectly on ULK1 as well. HOTAIR, as a typical example, targets AMPK/mTOR/ULK1 pathways to upregulate autophagy\textsuperscript{99}. PURPL enhances physical interaction with mTOR and ULK1 to promote the level of p-ULK1 (Ser757) mediated by mTOR, thereby inhibiting autophagy to promote cutaneous melanoma\textsuperscript{100}.

Besides, circRNAs are an emerging hotspot for the post-transcriptional regulation of ULK1. circRNAs composed of a covalently closed-loop structure, and characterized by the absence

Figure 3  Transcriptional and post-transcriptional regulation of ULK1. (A) Some genes, DNA-binding proteins, transcription co-activators and transcription factors regulate ULK1 expression. (B) Post-transcriptional regulation of ULK1 via RNA helicase, miRNAs, long non-coding RNAs and circRNAs.

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of 5’cap and 3’tail\textsuperscript{101}. Of them, CircCDYL, an autophagy-associated circRNA, promotes the development of breast cancer through miR-1275$\rightarrow$ATG7/ULK1 pathway and may serve as a promising predictive molecule of breast cancer\textsuperscript{44}. And in gastric cancer (GC), TMEM87A could elevate ULK1 via spiking miR-142-5p to contribute to cancer cell proliferation and metastasis\textsuperscript{102}. In addition, up-regulation of circ_0009910 was detected in the serum of imatinib-resistant chronic myeloid leukemia patients, and this up-regulation enhanced ULK1-regulated autophagy by targeting miR-34a-5p to promote drug resistance of cancer cells\textsuperscript{45} (Table 1, Fig. 3B). Therefore, post-transcriptional regulation has a significant impact on the expression and activity of ULK1, and elucidating the effect of post-transcriptional regulation on ULK1 will help to understand the importance of ULK1 in human diseases.

3.2. Post-translational modifications of ULK1 signaling network

A variety of proteins constitute an exquisite PPI network to regulate and be regulated by ULK1, involving multiple different signaling pathways and participating in a variety of life processes. Of note, these regulations are mainly post-translational modifications such as phosphorylation, ubiquitination, methylation, acetylation, glycosylation and sulfhydration (Fig. 4 and Table 2). In this section, we aimed to review the post-translational modifications of ULK1-network in canonical autophagy, non-canonical autophagy, mitophagy and non-autophagy functions.

3.2.1. Post-translational modifications of ULK1-network in canonical autophagy

3.2.1.1. Upstream pathways. ULK1, known as the initiator of canonical autophagy, many proteins control the process of autophagy by regulating its activity in various ways (Fig. 4A). Among them, the most well-known is the regulation of ULK1 activity by AMPK and mammalian target of rapamycin (mTOR) via phosphorylation. The cell energy sensor AMPK sustains cell energy balance by sensing the cell energy state and promotes autophagy; on the contrary, mTOR exerts an inhibitory effect in autophagy and is a regulator of cell growth and nutritional signal transduction. Under nutrient deprivation, AMPK could activate ULK1 via directly phosphorylation ULK1 at Ser317, Ser777, Ser555, Thr574, Ser637 and Ser647 to promote autophagy\textsuperscript{12,103,104}. While when nutrient sufficiency, ULK1 could be phosphorylated at Ser757 and Ser638 by mTORC1 directly to disrupt AMPK–ULK1 interaction to impede ULK1-induced autophagy activation further; interestingly, this signal may inactivate ULK1 by phosphorylation at Ser647\textsuperscript{105,106}. And ULK1 can phosphorylate TOR1-regulated protein (Raptor), which prevents mTOR from binding to substrate Raptor, and thus inhibiting the activation of mTORC1 signaling pathway\textsuperscript{12,106}. Intriguingly, ULK1 could affect S6K1 (substrate of mTORC1) Thr389-specific kinase or phosphatase in Drosophila and mammalian cells\textsuperscript{107}. In addition, ULK1 overexpression increases the phosphorylation of Raptor at Ser863, further critically inhibits phosphorylation of S6K1 and 4E-BP1 mediated by mTORC1, which reduce Raptor’s affinity binding to 4E-BPI’s substrate yet has little effect on the protein—protein interaction of the mTORC1 component\textsuperscript{108}. Under starvation, mitogen-Activated protein kinase 15 (MAPK15) directly stimulated AMPK-dependent ULK1 activity and affected the phosphorylation of ULK1 to activate ULK1\textsuperscript{106,109}. Besides, glycogen synthase kinase 3 (GSK3$\beta$) promotes the phosphorylation of ULK1 at Ser405 and Ser415 in gamma-aminobutyric acid receptor-associated protein (GABARAP)-interacting regions. Phosphorylation of these residues promotes the interaction of ULK1 with MAP1LC3B and gamma-aminobutyric acid receptor-associated protein-like 1 (GABARAPL1), thereby inducing autophagic flux\textsuperscript{110}. Moreover, PKC$\textalpha$ phosphorylates ULK1 Ser423 to prevent autolysosome formation, during which ULK1 does not alter activity but reduces autophagosome-lysosome fusion by binding to synapsin 17. Further, phosphorylated ULK1 promotes autophagy-mediated by chaperone promoting degradation of ULK1 by interacting with the heat shock cognate 70 kDa protein\textsuperscript{101,111}. However, WNK lysine deficient protein kinase 1 (WNK1) could inhibit autophagy via inhibiting phosphorylation of ULK1\textsuperscript{112,113}. Through phosphorylation proteomic analysis, combined with relevant experimental data, studies found that ULK1 can directly phosphorylate and promote the regulation of PP2A subunit striatum. Activated PP2A enhances autophagy through positive feedback regulation, promotes protein transport, and degrades proteins\textsuperscript{114}. Beside the above phosphorylation regulation, there are many other proteins that regulate the phosphorylation of ULK1 (Table 2), and contribute to the functions of ULK1.

In addition to phosphorylation regulation, ubiquitination regulation is also critical for ULK1 stability and activity. For instance, ubiquitin-specific protease (USP) 20 could inhibit the degradation of ULK1 by lysosomes through deubiquitylation and stabilization of ULK1, and play a positive role in autophagy initiation\textsuperscript{115}. Ubiquitin specific peptidase 24 (USP24) also inhibits autophagic activity by ubiquitinating ULK1 and affecting its stability. Not surprisingly, when USP24 was knocked out, the expression level of ULK1 was significantly increased and the autophagic flux was increased\textsuperscript{117}. However, USP1, as a key player in the modulation of ULK1 K63-linked deubiquitylation, regulates the Lys63 of ULK1, increasing normative autophagy flux and inhibiting alternative pathways, which lead to lysosomal-mediated degradation of sequestosome-1 (SQSTM1/p62)\textsuperscript{118}. In addition, the E3 ligase NEDD4L specifically ubiquitinates Lys925 and Lys933 sites of ULK1 for degradation by the proteasome to prevent cell death from excessive autophagy\textsuperscript{119}. TNF receptor associated factor 6 (TRAF6) ubiquitin ligase induces autophagy by ubiquitinating, stabilizing, and activating Lys63 of ULK1\textsuperscript{120}. Interestingly, AMBRA1 can also interact with TRAF6, ubiquitinate ULK1 with a Lys63-linked chain, strengthen its stability and regulate its function\textsuperscript{121}. When muscle cells atrophy, the ubiquitin ligase TRIM32 binds to AMBRA1 and ULK1 and activates ULK1 through unanchored K63-linked polyubiquitin chains\textsuperscript{122}. In addition, STAM-binding protein (STAMBP/AMSH) stabilizes ULK1 by eliminating K48-linked ubiquitination, thus positively affecting autophagy flux\textsuperscript{123}. Additionally, nerve growth factor (NGF) could control ULK1 activity via interacting with ULK1 to regulate its polyubiquitination\textsuperscript{124}. Besides, tripartite motif-containing protein 16 (TRIM16) could interact with ULK1 and promoted its K63-linked ubiquitination. Interestingly, ULK1 could in turn regulate TRIM6 activity via phosphorylation it at Ser16 and Ser203\textsuperscript{125}.

Notably, recent studies have indicated that methylation, acetylation, glycosylation and sulfhydration modifications are also important for the regulation of ULK1 activity. Recently, researchers found that the methylation of ULK1 is crucial for autophagy process under hypoxia\textsuperscript{126}. Mechanistically, protein arginine methyltransferase 5 (PRMT5) dimethylation of ULK1 at arginine 170 (R170me2s) could enhance the autophosphorylation of ULK1 at Thr180 to activate ULK1. While lysine demethylase 5C (KDM5C) could remove this modification via its demethylase activity to attenuate the activation of ULK1\textsuperscript{126}. Besides, HIV Tat-interactive protein (TIP60), an acetyltransferase, activates ULK1 to regulate autophagy flux in the face of growth factor deprivation.
The Ser86 site of the TIP60 is phosphorylated by GSK3, and TIP60 regulates its activity by acetylation of ULK1 at Lys162 and Lys606. Importantly, GSK3 operates the TIP60–ULK1 pathway connecting two different modifications of phosphorylation and acetylation, which is the ingenious and brilliant part of the regulation of cellular protein modification. Additionally, histone deacetylase 2 (HDAC2) could regulate ULK1 by deacetylation its K68 site, and this modification contributed to pyroptosis regulated by ULK1 in acute liver failure. Interestingly, when the glucose level is limited, the Thr754 site of ULK1 can be glycosylated by O-linked N-acetylglucosamine transferase (OGT), which is essential for the binding and phosphorylation of ATG14, thereby stimulating phosphatidylinositol-(3)-phosphate to promote the formation of phagocytes and initiates autophagy. Recently, sulfhydration modification was also found to regulate ULK1 activity and autophagy by cystathionine gamma-lyase (CSE) sulfhydration of ULK1 at Cys951.

3.2.1.2. Downstream pathways. Correspondingly, as the initiator of autophagy, ULK1 indisputably regulates many downstream proteins to perform multiple processes of autophagy, through various signaling pathways (Fig. 4A). For instance, ULK1 phosphorylates ATG14 at Ser29 through mTOR pathway, promotes lipid kinase activity of Vps34, and activates autophagy. Further,
Table 2  Post-translational modification of ULK1, the ULK1 complex and ULK1 regulated relevant pathways.

| Function          | The substrate protein | Modified site                  | Positive regulation/ negative regulation | Modification enzyme | Ref. |
|-------------------|-----------------------|--------------------------------|------------------------------------------|---------------------|------|
| Phosphorylation   | ULK1                  | Ser317, Ser777, Ser555, Thr574, Ser637, Ser467 | Activate                                  | AMPK                | 12,103,106 |
| Phosphorylation   | ULK1                  | Ser757/758, Ser658, Ser467      | Inhibit                                  | mTOR                | 103,104 |
| Phosphorylation   | mTOR                  | Ser2481                         | Inhibit                                  | ULK1                | 107  |
| Phosphorylation   | Sterk1                | Thr389                          | Inhibit                                  | ULK1                | 107  |
| Phosphorylation   | Raptor                | Ser863, Ser855, Ser859, Ser792  | Inhibit                                  | ULK1                | 107  |
| Phosphorylation   | ULK1                  | Ser317, Ser555                  | Activate                                 | MAPK15              | 108,109 |
| Phosphorylation   | ULK1                  | Ser405, Ser415                  | Activate                                 | GSK3β               | 110  |
| Phosphorylation   | ULK1                  | Ser423, Ser317/555/777          | Inhibit                                  | PKCα                | 111,112 |
| Phosphorylation   | ULK1                  | Ser555, Ser757                  | Inhibit                                  | WNK1                | 113,114 |
| Phosphorylation   | ULK1                  | /                               | Activate                                  | PP2A                | 115  |
| Ubiquitination    | ULK1                  | /                               | Stabilize                                 | USP20               | 116  |
| Ubiquitination    | ULK1                  | /                               | Destabilize                               | USP24               | 117  |
| Ubiquitination    | ULK1                  | /                               | /                                         | USP1                | 118  |
| Ubiquitination    | ULK1                  | Lys925, Lys933                  | Inhibit                                  | NEDD4L              | 119  |
| Ubiquitination    | ULK1                  | Lys63                           | Activate                                 | ULK1                | 112  |
| Ubiquitination    | ULK1                  | /                               | Activate                                  | TRAF6-AMBRA1        | 121  |
| Ubiquitination    | ULK1                  | /                               | Activate                                  | TRIM32-AMBRA1       | 122  |
| Deubiquitination  | ULK1                  | Lys48                           | Stabilize                                 | STAMBP/AMSH         | 123  |
| PolyUbiquitination| ULK1                  | K63                             | Activate                                  | NGF                 | 124  |
| Ubiquitination    | ULK1                  | K63                             | Activate                                  | TRIM16              | 125  |
| Dimethylation     | ULK1                  | Ser116, Ser203                  | Activate                                 | ULK1                | 125  |
| Dimethylation     | ULK1                  | Arginine170                     | Activate                                 | PRMT5               | 126  |
| Demethylation     | ULK1                  | Arginine170                     | Inhibit                                  | KDM5C               | 126  |
| Acetylation       | ULK1                  | Lys162 and Lys606               | Activate                                 | TIP60               | 127  |
| Deacetylation     | ULK1                  | Lys68                           | Inhibit                                  | HDAC2               | 128  |
| Glycosylation     | ULK1                  | Thr754                          | Activate                                 | OGT                 | 129  |
| Glycosylation     | ULK1                  | Cys951                          | Activate                                 | CSE                 | 130  |
| Phosphorylation   | ATG14                 | Ser29                           | Activate                                 | ULK1                | 131  |
| Phosphorylation   | Beclin-1              | Ser30                           | Activate                                 | ULK1                | 132  |
| Phosphorylation   | VPS15                 | Ser861, Ser865                  | Activate                                 | ULK1                | 133  |
| Phosphorylation   | mATG9                 | Ser14                           | Activate                                 | ULK1                | 134  |
| Phosphorylation   | DENND3                | Ser554, Ser572                  | Activate                                 | ULK1                | 135  |
| Phosphorylation   | PIKFYVE               | Ser1548                         | Activate                                 | ULK1                | 136,137 |
| Phosphorylation   | ULK1                  | Ser1042/Thr1046                 | Activate                                 | ULK1                | 139  |
| Ubiquitination    | ULK1                  | /                               | Activate                                  | KHL1L20             | 139  |
| Phosphorylation   | SEC23B                | Ser186                          | Inhibit                                  | ULK1                | 140  |
| Phosphorylation   | AMBRA1                | /                               | Activate                                  | ULK1                | 141  |
| Phosphorylation   | ATG4B                 | Ser316                          | Activate                                 | ULK1                | 142  |
| Phosphorylation   | FLCN                  | Ser406, Ser537, Ser54           | Activate                                 | ULK1                | 143  |
| Phosphorylation   | PPP2/PP2A             | /                               | Activate                                  | ULK1                | 144  |
| Phosphorylation   | ULK1                  | Ser317                          | Activate                                 | SQSTM1/p62          | 145  |
| Phosphorylation   | ULK1                  | Ser317                          | Activate                                 | PERK                | 146  |
| Phosphorylation   | PERK                  | Ser317                          | Activate                                 | ULK1                | 146  |
| Dephosphorylation | ULK1                  | Ser637                          | Activate                                 | PPM1D               | 147  |
| Phosphorylation   | ULK1                  | Ser556                          | Activate                                 | DAPK3               | 148  |
| Phosphorylation   | ULK1                  | Ser469, Ser495, Ser533          | Inhibit                                  | TOPK                | 149  |
| Phosphorylation   | ULK1                  | Ser504, Ser757                  | Inhibit                                  | p38 MAPK            | 150  |
| Phosphorylation   | ULK1                  | D485                            | cleave                                   | Caspase 3           | 151  |
| Phosphorylation   | SQSTM1/p62            | Ser409                          | Activate                                 | ULK1                | 152  |
| Phosphorylation   | ATG16L1               | Ser278                          | Activate                                 | ULK1                | 153  |
| Phosphorylation   | SEC16A                | Ser846                          | Activate                                 | ULK1/2              | 154  |
| Ubiquitination    | ULK1                  | /                               | Activate                                  | MUL1                | 155  |
| Phosphorylation   | ULK1                  | /                               | /                                         | MAPK1/3             | 156  |
| Phosphorylation   | ULK1                  | /                               | /                                         | NS1                 | 157  |
| Phosphorylation   | FUND C1               | Ser17                           | Activate                                 | ULK1                | 158  |
| Phosphorylation   | Rab9                  | Ser179                          | Activate                                 | ULK1                | 160  |
| Phosphorylation   | FII                   | Ser64                           | Inhibit                                  | ULK1                | 161  |
| Phosphorylation   | ULK1                  | /                               | Activate                                  | TBK1                | 163  |
| Phosphorylation   | TBK1                  | Ser172                          | Activate                                  | ULK1                | 164  |
| Phosphorylation   | ULK1                  | Ser774                          | Activate                                 | AKT                 | 165  |
| Ubiquitination    | ULK1                  | Lys48                           | Inhibit                                  | TRAF3               | 166  |

(continued on next page)
ULK1 phosphorylates Ser30 of beclin-1, and activates the PIK3C3 complex containing ATG14, thus promoting the formation of autophagosomes under amino acid starvation, hypoxia, and mTORC1 inhibition. Another member of the Vps34 complex, VPS15 can also be phosphorylated at Ser861 and Ser865 by ULK1, which enhance its autophagy-promoting ability. Under the circumstance of nutrient limitation, ULK1 phosphorylate Ser14 sites of mATG9, synergistically promote the interaction between mATG9 and adaptor protein 1/2; induce mATG9 autophagy vesicle transport; further initiating autophagy. Also, during starvation, ULK1 phosphorylates Ser554 and Ser572 sites of guanine nucleotide exchange factor DENN domain-containing protein 3 (DENND3), which increased its activity on GTGase Rab12, Activated Rab12 binds to LC3 to promote the transport of autophagosomes in starvation-induced autophagy. Additionally, ULK1 can phosphorylate and activate the lipid kinase PIKfyve Ser1548 in the face of intracellular glucose starvation, thereby promoting the formation of autophagosomes containing phosphatidylinositol 5-phosphate, thus driving the up-regulation of autophagy. ULK1 is also a substrate for Cul3-Klhl20 ubiquitin ligase. During starvation, ULK1 auto phosphorylation promotes the ubiquitination and proteolysis of the Kelch-like protein Klhl20, contributing to autophagy termination. What’s more, in the case of nutritional deficiencies, ULK1 phosphorylates SEC23B Ser186 to prevent the interaction of SEC23B with the repeat protein FBXW5, thereby stabilizing SEC23B. Phosphorylation and stabilization of SEC23B can enable its interaction with SEC24A and SEC24B, relocate to the ER-Golgi intermediate compartment, and further increase autophagy flux. Also, in ER, ULK1 phosphorylates AMBRA1 and regulates the dissociation of AMBRA1-DLC1 from the dynein complex, and of note, AMBRA1-DLC1 relocates to the ER when autophagy begins. In addition, ULK1 directly phosphorylates and inhibits human ATG4B on Ser316 to inhibit LC3 processing. Conversely, the phosphatase PP2A-P2R3B could eliminate this process. They cooperate to regulate the LC3 process by governing the cellular activity of ATG4B. Moreover, folliculin (FLCN) is closely related to the cellular pathway and is a novel autophagy element. The absence of FLCN will moderately damage the basic autophagy flux. ULK1 phosphorylates Ser406, Ser537, and Ser542 of the FLCN complex, which interacts with GABARAP, the second component of the autophagy machinery that also regulated by ULK1. Besides, based on unbiased phosphor-proteomic experiments, it was found that ULK1 phosphorylated the STRN subunit of protein phosphatase 2 (PPP2/PP2A), which in turn supports PPP2 activation through a positive feedback loop and increases protein degradation by promoting autophagy.

### Table 2 (continued)

| Function          | The substrate protein | Modified site | Positive regulation/ negative regulation | Modification enzyme | Ref. |
|-------------------|-----------------------|---------------|------------------------------------------|---------------------|------|
| Phosphorylation   | ULK1                  | Ser30         | Activate                                  |                     |      |
| Phosphorylation   | ULK1                  | /             | Activate                                  | p38α                | 167  |
| Phosphorylation   | LARS1                 | Ser391, Ser720| Inhibit                                   | ULK1                | 169,170 |
| Phosphorylation   | HK                    | Ser124, Ser364| Activate                                  | ULK1/2              | 171  |
| Phosphorylation   | PKK1                  | Ser74, Ser762 | Activate                                  | ULK1/2              | 171  |
| Phosphorylation   | ENO1                  | Ser115, Ser282| Activate                                  | ULK1/2              | 171  |
| Phosphorylation   | FBP1                  | Ser63, Ser88  | Activate                                  | ULK1/2              | 171  |
| Phosphorylation   | Dsh                   | Ser239, Ser247, Ser254, Ser266, Ser376, Ser554, Ser555| Inhibit             | ULK1                | 173  |
| Phosphorylation   | Exo70                 | Ser89         | Inhibit                                   | ULK1                | 174  |
| Phosphorylation   | Cdc37                 | Ser339        | Activate                                  | ULK1                | 175  |
| Phosphorylation   | Mad1                  | Ser546        | Activate                                  | ULK1                | 176  |
| Phosphorylation   | STING                 | Ser366        | Inhibit                                   | ULK1                | 178  |
| Phosphorylation   | RIPK1                 | Ser557        | Inhibit                                   | ULK1                | 180  |

### 3.2.2. Post-translational modification of ULK1-network in non-canonical autophagy

#### 3.2.2.1. Upstream pathways

ULK1 can also participate in regulating some non-canonical autophagy signaling pathways (Fig. 4B), such as stress, inflammation, toxic reactions, etc. SQSTM1/p62 induces phosphorylation of ULK1 at Ser504 and Ser757, promotes reciprocal regulation of AMPK and ULK1, and induces autophagy to degrade kelch like ECH associated protein 1 (KEAP1) and activate NFE2L2/NRF2, which rescues lipotoxicity in mouse liver cells. Also, PKR-like ER kinase (PERK) can directly phosphorylate ULK1 Ser317 and subsequently induce autophagic KEAP1 degradation in response to lipotoxicity. Notably, protein phosphatase 1D magnesium-dependent delta isoform (PPM1D) does not participate in the autophagy process induced by starvation, it regulates ULK1 activity by dephosphorylating ULK1 Ser637 in a p53-dependent manner and formats ULK1 puncta to activate autophagy.

Further, multiple proteins exert anti-tumor effects through phosphorylation of ULK1. Death-associated protein kinase 3 (DAPK3) can directly promote the formation of ULK1 complex by phosphorylation at Ser556, which contribute to the anticancer role of DAPK3 in GC. T-LAK cell-derived protein kinase (TOPK) could also inhibit the activity and stability of ULK1 by phosphorylating ULK1 (Ser469, Ser495, and Ser533), thereby reducing autophagic activity and decreasing the sensitivity to temozolomide of glioma. What’s more, p38/MAPK triggers the phosphorylation of ULK1 at Ser504 and Ser757, preventing its binding to downstream effector protein ATG13, inhibiting autophagy in microglia, and promoting microglial inflammation. Intriguingly, apoptotic executive protein caspase-3 could cleave ULK1 on D485 site thus to control ULK1 activity in leukemogenesis.

#### 3.2.2.2. Downstream pathways

Proteotoxic stress promotes phosphorylation of SQSTM1/p62 at the ubiquitin-association (UBA) domain of toxins, regulating its binding to ubiquitinated proteins.
proteins. Mechanistically, when nutrients are abundant, ULK1 phosphorylates SQSTM1/p62 at UBA Ser409, thereby destroying the stability of the UBA dimer interface as well as increasing SQSTM1/p62 binding to ubiquitin. ULK1 also activates autophagy by phosphorylation of downstream proteins to participate in the regulation of some stress mechanisms. Excepted for lapidating LC3B and driving the formation of autophagosomes, ATG16L1 is also a direct ULK1 target and regulated by ULK1 phosphorylation at Ser278 during stress. In addition, based on proteomic methods combined with experimental methods, it was found that ULK1 phosphorylated SEC16A at Ser846, promoting the assembling COPII components in ERES, and regulating the transport of ER to the Golgi apparatus.

3.2.3. Post-translational modification of ULK1-network in mitophagy

3.2.3.1. Upstream pathways. Notably, ULK1 also has a very important role in the selective autophagy of cells, of which mitophagy is the classical type (Fig. 4C). ULK1 often acts as an upstream regulator to regulate mitophagy, but there are still some proteins that can affect the process of mitophagy by affecting ULK1. For instance, in seledite-induced mitophagy, ULK1 acts as a substrate for mitochondrial E3 ubiquitin ligase 1 (MUL1). MUL1 ubiquitinates ULK1 and promotes ULK1 degradation to regulate seledite-induced mitophagy. Notably, functions of ULK1 in mitophagy also affect the disease’s progression of cancer, inflammation, and viral infections. For example, abnormal levels of ULK1 expression are frequently observed in breast cancer, which may be due to the degradation of Lys48-linked ULK1 ubiquitination by MAPK1/3 kinases in breast cancer. Not surprisingly, disrupting the MAPK1/3 kinases to increase the phosphorylation of ULK1 and inducing mitophagy, which works well in the treatment of bone metastases with low ULK1 levels. Nonstructural protein 1 (NS1) protein could also trigger the phosphorylation of ULK1 and enhances the mitochondrial expression of BCL2-interacting protein 3, which were vital for influenza virus mediated mitophagy.

3.2.3.2. Downstream pathways. To regulate mitophagy, ULK1 could increase the phosphorylation of FUND1C at Ser17, thereby enhancing the binding of FUND1C to LC3. Furthermore, the interaction of Sestrin-2 with ULK1 promotes ULK1-mediated phosphorylation of Beclin1 at Ser14, promoting Parkin translocation to the mitochondrial membrane and activating mitophagy. Further studies revealed that ULK1 phosphorylates receptor-interacting Ser/Thr protein kinase 1 Rab9 at Ser179, thereby recruiting Rab9-linked trans-Golgi membranes to impaired mitochondria. This is a pathway mediated by mitophagy that protects the heart from ischemia by degrading damaged mitochondria. Additionally, Flightless-I (FliI), a p62 interacting protein, was impeded by ULK1 phosphorylation at Ser64 while impairing ULK1, and the phosphorylation of this site is activated by insulin. ULK1 directly phosphorylation of critical glycolytic enzymes such as hexokinase (HK), phosphofructokinase 1 (PFK1), enolase enzyme 1 (ENO1) and gluconeogenic enzyme-1,6-bisphosphatase (FBP1), which further activated the glycolytic pathway to maintain glucose metabolism. Of note, Disheveled (Dsh) plays a central role in the Wnt/β-catenin pathway, which is a key pathway that determines the differentiation fate of cells during development. Interestingly, ULK1 can phosphorylate Dsh at multiple sites, such as Ser239, Ser247, and Ser254 in PDZ-DEP, as well as Ser239, Ser247, Ser254, Ser256, Ser376, and Ser554. Moreover, ULK1 is of great importance in cancer-related signaling pathways. For instance, the exocyst subunit Exo70 is phosphorylated by ULK1 at the Ser89 site and thus inhibits cancer cell metastasis. Additionally, ULK1 mediates phosphorylation of co-chaperone cell division cycle protein 37 (CDC37) at Ser339, which impairs CDC37-coordinated HSP90 function and contributes to degrading HSP90–CDC37 client kinases, thereby increasing the lethality of HSP90 inhibitors to tumor cells. Further, Mad1, the spindle assembly checkpoint protein, is phosphorylated by ULK1 at Ser546 for recruitment of Mad1 to kinetochore to maintain chromosome fidelity during cancer cell mitosis. Importantly, ULK1 also involved in the regulation of several inflammatory and stress related pathways. VCP/p97 can be phosphorylated at Ser13, Ser282, and Thr761 by ULK1/2 localized to stress granules to treat VCP mutation-induced IBM-like disease by promoting VCP activity and disaggregating stress granules. Circulating dinucleotide could also promote the function of the stimulator of interferon genes (STING), which transports TBK1 to the endosome/lysosome and activates interferon regulatory factor 3 as well as nuclear factor 1 progenitor eB (NF-eB). ULK1 was proved to phosphorylated STING at Ser366, which subsequently prevented the persistent transcription of innate immune genes. Strikingly, ULK1 is also involved in the regulation the necroptosis pathway. The receptor-interacting kinase 1 (RIPK1) is a key mediator of TNF-induced signaling, in the process of cell life, RIPK1 acts as the gatekeeper of cell life and death. Particularly, it can decide the destiny of the cell, and the way of cell death. Intriguingly, ULK1 could...
phosphorylation of RIPK1 at Ser357, which impeded RIPK1-mediated cell death \(^{180}\).

### 3.3. Other regulation of ULK1

Interestingly, in addition to transcriptional regulation, post-transcriptional regulation, and post-translational regulation, there are many other regulators that can affect the function and activity of ULK1 (Table 3). Metal copper (Cu), which always acts as the static cofactor within enzyme active sites, was found to contribute to the activation of ULK1/2 through direct interaction. And this interaction was associated with ULK1-mediated autophagosome formation and the autophagy progression \(^{181}\). Intriguingly, researchers found GABARAP and GABARAPL1 could co-regulate ULK1 activity with LC3B/C. Mechanistically, GABARAP and GABARAPL1 bind to ULK1 and ATG13 and enhance ULK1 activity to promote autophagy. On the contrary, LC3B and LC3C decrease the activity of ULK1 through inhibiting GABARAPL1 lipation \(^{182}\). Moreover, some regulators can affect the activity of ULK1 by regulating the localization of ULK1. For instance, the protein TFG encoded by Trk fusion gene (TGF) plays a pivotal role in autophagy initiation by interacting with ULK1, increasing the LC3C–ULK1 binding \(^{183}\). S100A10, a member of the S100 protein family, was found to directly interact with ULK1 and further stimulate the localization of ULK1 to ER-mitochondria contact sites \(^{184}\). Interestingly, hypoxia can stimulate ULK1 to translocate into nucleus, further regulating YAP-driven glycolysis in pancreatic ductal adenocarcinoma (PDAC) \(^{185}\). Additionally, some regulators enhance the interaction of ULK1 with its partner to affect ULK1 and autophagy. C9orf72, whose mutations are the most common genetic causes for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), was proved to function as a component of the ULK1 complex by directly interacting with ATG13 in the ULK1 complex \(^{186}\). The adaptor NDP52 triggers membrane recruitment of the ULK1 complex to initiate mitophagy \(^{187}\). And Rab GTPase SMCR8 could negative regulation of ULK1 kinase activity via binding to ULK1 complex components \(^{188}\). GTases RAB2 can also interact with the ULK1 complex, thereby promoting the recruitment of the ULK1 complex to form autophagosomes \(^{189}\). Interestingly, NPM1 mutant NPM1-ma could interact with ULK1 protein and positively regulate ULK1 protein levels and contribute to leukemia cell survival \(^{190}\). IRGM, encoded by a human gene for inflammatory disease risk, plays anti-inflammatory roles by promoting the interaction and co-assembly of ULK1 and Beclin 1 and the formation of the ULK1 complex \(^{191}\). What’s more, the Golgi protein SCOC can interact with the ULK1-binding protein fiber bundles and the elongation protein zeta 1 (FEZ1). Subsequently, SCOC, ultraviolet radiation-related genes, and FEZ1 form a starvation-sensitive trimeric complex that regulates the activity of ULK1. Of note, during glucose starvation, SCOC promotes autophagy by stabilizing the ULK1–FEZ1–SCOC complex \(^{192}\). Atlastin 2 and 3 (ATL2/3), as ER-localized transmembrane proteins, could recruit and stabilize ULK1 and ATG101, thereby transporting to ER to form autophagosomes designated by FIP200–ATG13 \(^{193}\). Besides, TRIM5α is an antiretroviral protein that has been shown to interact with upstream proteins required to initiate autophagy, such as ULK1, to protect cells from infection. Mechanistically, TRIM5α interacts with ULK1 and affected cytoplasmic distribution of active ULK1 (p-ULK1Ser317) \(^{194,195}\). Intriguingly, cytosolic Malate dehydrogenase 1 (MDH1) has been shown to indirectly regulate ULK1 via regulation of its proteasomal degradation, and when autophagy was activated, the activity of MDH1 could be in turn activated \(^{196}\). And a member of the claudin family protein CLDN1, nucleoporin TPR and metastasis-associated colon cancer-1 (MACC1) were proved to indirectly regulate ULK1 through AMPK and mTOR pathway \(^{197,198}\). Moreover, RNA m7G methyltransferase complex proteins METTL1 was found to facilitate tumorigenesis through RPTOR–ULK1 axis. Mechanistically, RPTOR, which is a regulatory associated protein of mTOR complex 1, regulated ULK1 activity through mTOR phosphorylation \(^{180}\) (Fig. 5).

In short, the transcriptional regulation, post-transcriptional regulation, post-translational regulation, and other regulatory methods of ULK1 constitute a complex and exquisite cellular program with ULK1 as the core. Through the in-depth exploration of these regulation, we can understand the important role of ULK1 and its precise molecular mechanisms.

### 4. Autophagic and non-autophagic functions of ULK1 in human diseases

Since the multiple biological functions of ULK1, and ULK1-related signaling pathways have extensive effects on autophagy and other critical cellular processes, its dysregulation can lead to malignancies, neurodegenerative diseases, obesity, heart disease, infections, and other diseases by altering normal human physiology. In this section, we will detail how ULK1 affects the progression of various diseases through its autophagic and non-autophagic functions (Fig. 6).

#### 4.1. Cancers

##### 4.1.1. Autophagic function

Autophagy shows a double-edged sword role in different types, stages, and genetic contexts of cancers. In the early phases of tumorigenesis, activation of autophagy might prevent cancer development \(^{201}\). On the contrary, in advanced cancer, both autophagy enhancement and autophagy inhibition have been proposed as therapeutic strategies \(^{201}\). The potential role of autophagy in cancer is of extreme complexity and is always content-dependent, which deserves further discussion \(^{202}\).

On the one hand, autophagy controls the stability of genes and participates in the occurrence, development, treatment, and prognosis of tumors, thereby inhibiting tumors \(^{203}\). Particularly, in tumor tissues with reduced ULK1 expression, promoting autophagy-related cell death by activating ULK1 may be a prospective strategy. Of note, breast cancer is such a typical example, as the expression of ULK1 is remarkably down-regulated, and the baseline of autophagy is reduced as well, indicating that ULK1 might be a promising prognostic biomarker in breast cancer \(^{204}\). The Cancer Genome Atlas (TCGA) and Tissue Microarray (TMA) also exhibit significantly reduced ULK1 expression in breast cancer tissues, especially triple-negative breast cancer (TNBC) \(^{15}\). Mechanistically, the AMPK–ULK1–autophagy pathway correlates with tumor glycolysis orchestrates and tumor immunosuppression maintaining, which are associated with poor human TNBC outcomes \(^{205}\). In addition, ULK1-regulated mitophagy was confirmed to involved in the therapeutic response to targeted drug in breast cancer. For instance, the MAP2K/MEK inhibitor trametinib can up-regulated ULK1 to stimulate mitophagy, which contribute to the reduction of bone metastasis \(^{15}\). Therefore, activation of ULK1 is likely to be a brilliant therapeutic strategy for breast cancer.

On the other hand, autophagy can maintain the protection, survival, and defense mechanisms of cancer cells by keeping...
mitochondria functional, reducing DNA damage, enhancing cancer cell survival and anti-stress ability, etc., thereby maintaining tumor survival, ultimately promoting tumorigenesis, and causing resistance to therapeutic agents. Correspondingly, overexpression of ULK1 is negatively correlated with various cancers, including colon cancer, pancreatic ductal adenocarcinoma, liver cancer, prostate cancer (PC), chronic myelomonocytic leukemia, and glioblastoma. Studies have demonstrated that in colon cancer cells, after silencing eukaryotic elongation factor-2 kinase, activating autophagy promotes colon cancer cells survival via AMPK-ULK1 pathway, indicating that the down-regulation of ULK1 might constitute a new treatment for human colon

Table 3 Other factors regulation of ULK1.

| No. | Name       | Classification       | Positive regulation/ Negative regulation | Mechanism                                                                 | Ref. |
|-----|------------|----------------------|-------------------------------------------|---------------------------------------------------------------------------|------|
| 1   | Copper     | Metal ion            | Positive regulation                       | Enhancing ULK1 kinase activity by directly interaction                    | 181  |
| 2   | GABARAP/GABARAPL1 | Protein              | Positive regulation                       | GABARAP/GABARAPL1 positively regulate starvation-induced ULK1 activation and are important for maintaining the expression or stability of the ULK1 complex proteins | 182  |
| 3   | LC3B/C     | Protein              | Negative regulation                       | LC3B/C might negatively regulate ULK1 by reducing the expression or stability of GABARAPs | 182  |
| 4   | TFG        | Protein              | /                                        | Binding LC3C to regulate ULK1 localization and autophagosome formation     | 183  |
| 5   | S100A10    | Protein              | Positive regulation                       | S100A10 regulates ULK1 translocation to ER-mitochondria contact sites in response to IFN-stimulation | 184  |
| 6   | Hypoxia    | Cellular microenvironment | Positive regulation                 | Hypoxia stimulates ULK1 to translocate into nucleus                        | 185  |
| 7   | c9orf72    | Protein              | Positive regulation                       | C9orf72 regulates ULK1 expression via regulation of the ULK1 complex       | 186  |
| 8   | NDP52      | Protein              | Positive regulation                       | NDP52 recruits the ULK1 complex and triggers membrane recruitment          | 187  |
| 9   | SMCR8      | GTPases              | Negative regulation                       | SMCR8 regulates ULK1 kinase activity, ULK1 gene and protein expression     | 188  |
| 10  | RAB2       | GTPases              | Positive regulation                       | RAB2 facilitates the recruitment of ULK1 complex and modulates ULK1 kinase activity | 189  |
| 11  | NPM1-mA    | Protein              | Positive regulation                       | NPM1-mA promoted TRAF6-dependent K63 ubiquitination and further maintained ULK1 stability and kinase activity via miR-146a | 190  |
| 12  | IRGM       | Protein              | Positive regulation                       | IRGM interacts with ULK1 and Beclin 1 and promotes their co-assembly thus governing the formation of autophagy | 191  |
| 13  | SCOC       | Protein              | Positive regulation                       | Activating ULK1 via stabilizing ULK                                         | 192  |
| 14  | ATL2/3     | Protein              | Stabilize                                 | Stabilizing the ULK1 complex                                               | 193  |
| 15  | TRIM5α     | Protein              | Positive regulation                       | TRIM5α interacts with ULK1 and affected cytoplasmic distribution of p-ULK1 (Ser317) | 195  |
| 16  | MDH1       | Protein              | Positive regulation                       | MDH1 may regulate ULK1 proteosomal degradation                             | 196  |
| 17  | CLDN1      | Protein              | Positive regulation                       | CLDN1 increases ULK1 expression through AMPK/STAT1 signaling pathway       | 197  |
| 18  | TPR        | Protein              | Negative regulation                       | TPR regulates ULK1 activity via mTOR                                         | 198  |
| 19  | MACC1      | Protein              | Positive regulation                       | MACC1 regulates ULK1 via AMPK                                              | 199  |
| 20  | METTL1     | Protein              | Negative regulation                       | METTL1 regulates ULK1 via RPTOR, a regulatory associated protein of mTOR complex | 200  |
Indeed, drugs, such as Cordyceps Sinensis polysaccharide and curcumin combined with 5-FU, have been proved to suppress colon cancer cell proliferation by inhibiting autophagy and apoptosis via impairing AMPK/ULK1 pathway while blocked autophagy flux and lysosome formation. And down-regulating ULK1 could repress tumorigenic activities of pancreatic cancer cells by regulating autophagy-mediated mitochondrial metabolism. In the hypoxic microenvironment of PDAC, ULK1-triggered autophagy and glycolysis act as a niche for malignant growth and drug resistance by stabilizing previously unrecognized crosstalk between nuclear YAPs during PDAC development. Notably, PVT1/miR-20a-5p/ULK1-autophagy pathway, MDH1–ULK1 pathway, and LKB1–AMPK–ULK1 signaling axis, may be novel targets for developing therapeutic strategies for PDA by leading to autophagy induction. Besides, based on immunohistochemical analysis of TMAs, ULK1 combination with LC3B is the main prognostic factor for HCC patients, and ULK1-mediate autophagy was activated when HCC cells faced nutrient deprivation. In addition, alterations in mitochondrial DNA impairs ROS–AMPK–ULK1 signaling pathways, activating autophagy in HCC cells. Here, XST-14, a ULK1 inhibitor discovered by molecular docking, can block autophagy, induce apoptosis, and inhibit the development of HCC cells. Additionally, androgen receptor regulates ULK1 expression in PC patients. Experiments have shown that knocking down ULK1 inhibits androgen-mediated autophagy and PC cell proliferation. Moreover, high levels of ULK1 protein in primary PC are positively related to increased biochemical recurrence, both of which highlight the potential of targeting ULK1-mediated autophagy in the treatment of advanced PC. Besides, ULK1-mediated pro-survival autophagy can inhibit leukemia cell death in FLT3-ITD mutant leukemia, thus ULK1 may serve as a viable drug target for the treatment of FLT3-ITD mutant leukemia. Intriguingly, the mRNA expression levels of ULK1 in acute myeloid leukemia (AML) samples were analyzed based on TCGA database, and the level of ULK1 is elevated in AML, which was also associated with poor prognosis in AML. Moreover, in chronic myeloid leukemia (CML), inhibition of ULK1 could sensitize leukemic stem cells to tyrosine kinase inhibitor (TKI). Similarly, P2RY6-mediated autophagic activation of CAMKK2/PRKAA1/ULK1 restores human monocyte differentiation, which is exciting for chronic myelomonocytic leukemia therapy. In addition, autophagy is also crucial for glioma initiation and growth, destruction of ULK1 by RNAi could inhibition of autophagy and significantly reduced glioblastoma development, indicating that ULK1-mediated autophagy contributes to the survival of tumor. Notably, ULK1 levels (mRNA and protein) were remarkably increased in GC tissues. The high expression of ULK1 in GC was proved to related to the patient’s classification and cancer recurrence. Thus, the overexpression of ULK1 in GC

Figure 5. Other factors that regulation of ULK1-related PPI network. (A) Representative factors regulate ULK1 activity through directly interaction. (B) Representative factors regulate ULK1 activity through regulation of the ULK1 complex. (C) Representative factors regulate ULK1 activity through influence the subcellular localization of ULK1. (D) Representative factors regulate ULK1 activity through regulation of AMPK/mTOR. (E) Representative factors regulate ULK1 activity in some human disease.
decreases the survival of GC cells and increases the recurrence of cancer. And ULK1 may function as a prognostic biomarker for GC. In addition, the TCGA data of clear renal cell carcinoma (ccRCC) shows that the mRNA level of ULK1 was much higher in ccRCC tissues than normal kidney tissues. Inhibition of ULK1 by shRNA or ULK1 inhibitors induces cell apoptosis in ccRCC cells, which leading to anticancer efficacy in vivo. Importantly, AMPK–ULK1–LC3B axis contributes a lot to autophagy inhibition induced anti-cancer effects. Of note, with the extension of the treatment cycle, drug resistance in cancer cells is generally inevitable, and ULK1-regulated autophagy also plays a crucial role in drug resistance. For instance, drug resistance of the bromodomain and extraterminal domain inhibitor JQ1 in AML may result from the induction of protective autophagy in leukemia stem cells by activating the AMPK/ULK1 pathway. In addition, ULK1-mediated autophagy is one of the causes of drug resistance in TKI treatment of CML, which mediated by maintaining energy and redox balance. Therefore, inhibiting ULK1-induced stress differentiation of leukemia stem cells is a practical approach to enhance the therapeutic effect of TKI on CML. Moreover, imatinib resistance in the treatment of CML may be caused by granulacin activation of ULK1-mediated autophagy. Besides, osteosarcoma is prone to drug resistance after chemotherapy, attributed to the autophagy induction by enhancing the interaction of high mobility group box 1 protein with Beclin 1 mediated by the ULK1 complex.

4.1.2. Non-autophagic function

Similarly, the non-autophagy related functions of ULK1 also play a role in cancer. As mentioned in the preceding text, increased expression of ULK1 was negatively correlated with breast cancer metastasis by activating autophagy. Additionally, ULK1 also phosphorylates ERK1/2, which further phosphorylates Exo70, thereby inhibiting the invasion of human breast cancer cells, achieved from a non-autophagic route. In addition, ULK1 expression was found to correlate positively with the degree of aggressiveness of the tumor. The spindle checkpoint protein Mad1 is phosphorylated by ULK1, and then p-Mad1 may recruit kinetochores to promote proper mitotic progression, aligns, and segregates faithful chromosomes. Furthermore, evidence shows that ULK1 in cancer enhances the chromosomal stability of paclitaxel and reduces cytotoxicity, thereby promoting tumor cell growth.

In short, ULK1-mediated autophagy plays different roles in different tumor types and different conditions, acting as the criminal or savior. In general, ULK1-mediated autophagy suppresses cancer in the early stages of tumors while maintaining tumor growth in the late stage of the tumors. Besides, in a minority of advanced cancers, ULK1 also mediates metastasis-related signaling pathways to inhibit cancer invasion, which is a role of ULK1 beyond autophagy. Therefore, based on the complex role of ULK1 in cancer, it is necessary to further clarify the exact role of ULK1 in different situations and carry out corresponding studies.
interventions, so that the regulation of ULK1 may play a correct and brilliant role in cancer.

4.2. Neurodegenerative diseases

4.2.1. Autophagic function

Autophagy is closely related to a variety of neurodegenerative disorders, including Alzheimer’s disease (AD), PD, Huntington’s disease (HD), ALS, frontotemporal lobar degeneration, etc. The currently recognized pathogenesis of AD is mainly β-amyloid (Aβ) deposition and neurofibrillary tangles, followed by impaired blood–brain barrier function and neuroinflammation, which may be related to abnormal autophagy. Activation of autophagy can effectively reduce AD-related pathology. Of note, studies found that through inhibition of PI3K/Akt/mTOR/ULK1 pathway and effectively reduce AD-related pathology. Of note, studies found that through inhibition of PI3K/Akt/mTOR/ULK1 pathway and NQO1/mTOR/ULK1 pathway, as well as activation of AMPK/ULK1 pathway, Aβ load and plaques in the brain of AD mice can be reduced, and neuroinflammation and inflammatory bodies can be suppressed, thus improving learning and memory impairment. In addition, impaired steps in autophagy may contribute to PD, and peripheral blood mononuclear cells from PD patients exhibit down-regulated autophagy genes including ULK1 and elevated levels of α-synuclein, suggesting that activation of autophagy may have potential therapeutic effects in PD patients with low basal autophagic activity. Intriguingly, the ULK1 activator BL-918 indeed showed considerable therapeutic potential in cell and animal models of PD. Additionally, HD is a disease mainly caused by mutations in HTT (huntingtin protein) encoding polyglutamine (polyQ). Evidence suggests that mTOR-mediated ULK1 phosphorylation of ATG14 promotes Vps34 lipid kinase activity to clear polyQ mutants in a mouse HD model. Furthermore, ULK1 phosphorylates SQSTM1/p62 in the UBA domain, and next involves in the clearance of ubiquitinated proteins or polyQ-Htt. Moreover, multiple studies have demonstrated that C9orf72 deficiency or mutation will cause FTD and ALS disease, which is also related to ULK1. Mechanistically, C9orf72 can synergize with Rab1a and ULK1 to increase autophagic flux and reduce the accumulation of p62-positive puncta, which may be a relevant pathology in ALS/FTD. Besides, C9orf72 protein can also form complexes with SMCR8, WD repeat domain 41 (WD41), and ATG101 to regulate autophagic flux and restore neuronal morphology by regulating ULK1 to regulate autophagy. Additionally, ULK1 also exerts a pathogenic effect in the course of neurodegenerative disease. Chorea-acanthocytosis, an inherited neurodegenerative disease, is characterized by circulating acanthocytes lacking choline. Recent studies have reported that the level of ULK1 and ATG7 is increased in the cytoplasm of chorea-acanthocytosis erythrocytes because HSP forms an HSP90-70 macromolecular complex with ULK1 that sequesters toxic Lyn and hinders its transport, delaying mitochondrial and lysosome clearance.

4.2.2. Non-autophagic function

Importantly, beyond the autophagy induction role of ULK1, it can also affect neurodegenerative diseases through other ways. ULK1 was found to regulate the process of neuronal development, and its loss of function would contribute to neurodegeneration. Notably, studies have found that ULK1 play a positive role in neuroprotection and regeneration in the central nervous system. The expression of ULK1 is up-regulated in the hypothalamus after traumatic brain injury in mice, and knockout of ULK1 can improve the survival of hypothalamic neurons, reduce neuroinflammation and neuronal apoptosis, and reduce glial cell proliferation by inhibiting p38/JNK pathway. Porcine hemagglutinating encephalomyelitis virus (PHEV) is a neurotropic coronavirus that induces neurodegenerative diseases by invading the central nervous system of sucking piglets. A recent study demonstrated that ULK1 functional inhibition was involved in the regulation of PHEV-induced neuronal degeneration, manifested by hypoplastic axonal extension, increased dendritic branching, unstable dendritic spine formation, etc. PHEV infection significantly downregulated the phosphorylation level of the NGF high-affinity receptor TrkA and inhibited ULK1-mediated internalization of NGF, and induced neuronal damage. The up-regulation of mir-142a-5p caused by PHEV infection also negatively regulates NGF signal transduction by inhibiting the expression of ULK1, causing axonal transport disorder and inducing neurodegenerative diseases. It was also found that intracellular protein imbalance can lead to various neurodegenerative diseases, and ULK1 knockout mice exhibit obvious neuronal degeneration. Mechanistically, ULK1 can regulate ER-to-Golgi cargo transport through phosphorylation of SEC16a to maintain neuronal homeostasis. In addition, schizophrenia is a grievous, highly inherited mental disease. Based on exome sequence data, exome sequencing of patients with schizophrenia revealed four nonsense mutations in the ULK1 gene, pending independent validation by experiments. ULK1 also regulates repositioning of the axonal initial segment, which affects potential action generation and axonal polarity.

Therefore, ULK1 plays a protective role in most neurodegenerative diseases. Mechanistically, ULK1-mediated activation of autophagy can effectively reduce most pathologies associated with neurodegenerative diseases by clearing toxic proteins and reducing toxic proteins neuroinflammation and neuronal apoptosis to lessen brain damage and protect dopamine neurons. However, under certain conditions, ULK1 can play a pathogenic role by blocking the transport of toxic substances.

4.3. Immune-related diseases

4.3.1. Autophagic function

ULK1-mediated autophagy is essential in regulating immune responses, especially against virus infection. Naturally, autophagy acts as a mighty host defense mechanism that inhibits HIV replication. After HIV infection in Jurkat cells and CD4⁺ T cells, autophagy is induced by increasing the transcription of ULK1 to defend against HIV invasion. Invading microbial pathogens also can be selectively eliminated by xenophagy. Of note, TBK1 and NDP52 can recruit the ULK1 complex to cytoplasmic bacteria to initiate xenophagy and resist microbial pathogens’ invasion. Likewise, autophagy can limit the replication of herpes simplex virus 1 in host cells. Unfortunately, to counteract organism defense, HSV-1 Us3 Ser/Thr kinase cooperates with ICP34.5 to regulate the phosphorylation of ULK1 in virus-infected cells to inhibit autophagy and promote HSV replication. Salmonella enterica could cause severe gastroenteritis, and autophagy-mediated by Atg5 and ULK1 in host cells can resist to the invasion of the virus. However, Salmonella could still withstand the immune response by protecting itself from autophagy. Mechanistically, Salmonella virulence factors SseF and SseG can inhibit Rab1A, thereby reducing ULK1 recruitment and activation, impairing autophagy initiation. Brucella can utilize the Brucella vacuole (BCV) to send it into the ER of the host cell for...
replication to evade clearance by intracellular autophagy. BCV can be transformed into an autophagy-characterized compartment (aBCV), a process that requires activation of autophagy-initiating proteins such as ULK1, thereby forming the intracellular circulation of Brucella and facilitating subsequent infection of host cells.

Notably, in some cases viruses or pathogens can take advantage of autophagy-related proteins or products of host cells to assist their replication. For instance, human immune-related GTPase M (IRGM) promotes the co-localization of Golgi vesicles with replicating hepatitis C virus and induces autophagy by promoting ULK1 phosphorylation through IRGM, utilizing cell membrane remodeling, thereby promoting hepatitis C virus replication. Cryptococcus neoformans, a fungal pathogen that could induce the phosphorylation of LKB1 in host cell, promoted the phosphorylation of autophagy-initiating proteins, including ULK1, which play a role in C. neoformans internalization, trafficking, and replication. The miR-99 family activates autophagy via IGF-1R-mediated mTOR/ULK1 pathway to promote the replication of hepatitis B virus (HBV). Activated AMPK/mTOR–ULK1–autophagy axis promotes HBV replication at low glucose concentrations and predisposes hepatocytes to HBV infection. In lung inflammation and even acute lung injury under influenza A virus infection, lacking of HIF-1α decreases glycolysis levels and promotes autophagy through the AMPK/ULK1 pathway to promote IAV copy. Moreover, under infectious conditions, ULK1 kinase, can directly phosphorylate ATG16L1, and phosphorylated ATG16L1 localizes to the site of internalizing bacteria and promotes antimicrobial autophagy, a process implicated in Crohn's disease, and the mechanism remains to be investigated.

4.3.2. Non-autophagic function

Interferons are secreted cytokines to defend against viral infections, and ULK1 could regulate type I interferon (IFN)-dependent immunity. Mechanically, ULK1 is phosphorylated and activated through the involvement of type I IFN receptor at ser757, which is dependent on Akt activity, and then activated ULK1 regulates the expression level of IFN-stimulated genes by activating the p38 MAPK pathway, thereby regulating interferon signaling. IFN-γ receptor stimulation could also activate ULK1, and the interaction between ULK1 and the mixed lineage kinase 3 was necessary for mixed lineage kinase 3 phosphorylation and downstream activation of the kinase ERK5. ULK1 promotes the transcription of key antiviral IFN-stimulated genes and is essential for IFN-γ-dependent antiviral effects, which might be a vital element of the antiviral response. In addition, STING can detect abnormal DNA in the nucleus and directly transcribe and activate type I IFN and NF-κB. Importantly, when persistent DNA damage occurs, ULK1 could inhibit STING activity and reduce the expression of type I IFN by phosphorylating STING at Ser366. Additionally, ULK1/2 inhibits the formation of SIN1 puncta and then phosphorylates TBK1, which affects viral infections or protein aggregation diseases. Besides, the TFG forms a complex with the E3 ligase, TRAF3, and participates in the immune response by destroying the ULK1–TRAF3 interaction to stabilize ULK1 and protect macrophages from pyroptosis.

Thus, ULK1-mediated autophagy is a mechanism by which the host resists the invasion of viruses or pathogens. Nevertheless, some viruses or pathogens can also employ the autophagy-related proteins or products of host cells (including ULK1) to promote their replication. In addition, ULK1 can directly mediate some proteins to resist infection caused by viral invasion by non-autophagic pathways.

4.4. Cardiovascular diseases

4.4.1. Autophagic function

Autophagy is an important way for most cardiovascular-derived cells to maintain homeostasis and is a necessary condition for regulating cardiovascular function. Most heart-related diseases, including cardiovascular diseases, myocardial ischemia (IR), are generally accompanied by abnormal autophagy, which might be caused by mitochondrial dysfunction and oxidative stress. It is worth noting that autophagy has complex functions in IR. Lipocalin-2, a protein positively associated with heart failure, was found to downregulate the phosphorylation of AMPK and ULK1 to reduce autophagic flux, inhibit beneficial autophagy during IR, and increase ischemia-induced cardiomyocytes death. Notably, excessive autophagy aggravates IR damage. Currently, inhibiting autophagy by activating the Akt/mTOR/ULK1 pathway and inhibiting oxidative stress and cardiomyocyte apoptosis contributes to cardiomyocyte survival.

Intriguingly, there is a dramatic down-regulation of autophagy and levels of ULK1 in obesity-related cardiac dysfunction. High-fat diet feeding has no effect on triglyceride or diacylglycerol levels or cardiac function when Ulk1 and lipoprotein lipase are knocked out in mice. Therefore, obesity-induced cardiomyopathy can be treated by activating ULK1-mediated autophagy to clear proteinosis. For heart damage caused by certain drugs, ULK1-mediated autophagy could always protect the heart. For example, the beneficial effect of isiduniol on DOX-induced myocardial damage depends on AMPK and ULK1 phosphorylation. AMPK/mTOR/ULK1-mediated autophagy activation could slow disease progression in a model of isoproterenol-induced myocardial fibrosis. In glucose-induced myocardial injury, the autophagy induction of mTOR/ULK1 plays a pivotal role. Additionally, experiments show that high glucose can increase ROS, phosphorylation of mTOR and its phosphorylation of ULK1 in H9c2 cardiomyocytes, further inhibiting autophagy and inducing apoptosis. Thus, mTOR-induced inactivation of ULK1 inhibits autophagy and protects cardiomyocytes from high glucose toxicity. However, phosphorylation of ULK1 is not modulated by acute hyperglycemia. Acute ethanol toxicity could activate AMPK and promote autophagy, triggering myocardial contractile dysfunction. Of note, this process is achieved by inhibiting mTORC1 and ULK1 phosphorylation. In the abnormality of myocardial contraction induced by low ambient temperature, the phosphorylation of the autophagy-inhibiting signaling molecules Akt and mTOR decreases, while the phosphorylation of ULK1 increases, thereby causing an increase in cardiac autophagy.

Interestingly, long-term moderate exercise can produce benign changes in myocardial autophagy and protect the myocardium. Some scholars conducted long-term endurance exercise intervention on 8-week-old BALB/c mice. The experimental results showed that exercise training could significantly increase the expression and phosphorylation level of LC3II, Atg12, p62, and ULK1 (Ser555) proteins, protect cardiomyocytes, and maintain cardiac function. The AMPK/mTOR/ULK1 pathway is more critical in exercise-regulated autophagy. Different forms of exercise have various regulations on autophagy pathways. For example, endurance exercise can activate AMPK signaling pathway, inhibit mTOR,
activate ULK1, and finally trigger autophagy. In response to cardiac pressure overload, ULK1 also participates in the initiation of mitophagy, thereby protecting the heart from cardiac insufficiency. Furthermore, ULK1-Rab9-mediated mitophagy was activated during the chronic phase of high-fat diet depletion and rescued obese cardiomyopathy by controlling mitochondrial mass.

4.4.2. Non-autophagic function

More recently, the non-autophagic role of ULK1 may affect heart-related diseases, which enriches its broader role beyond autophagy in cellular signaling pathways. Recent studies have found that deletion of ULK1 in the adult heart accelerates the progression of cardiomyopathy and heart failure, interestingly not by affecting the ULK1-mediated autophagy pathway. In addition, ROS can affect the progression of various cardiac diseases, including cardiac hypertrophy, myocardial contractility disorders, cardiac extracellular matrix remodeling, and arrhythmias. Therefore, the reduction of oxidative stress is probably an effective preventive measure against stress overload-induced cardiac hypertrophy. Intriguingly, studies have shown that ULK1 levels are elevated in dilated cardiomyopathy. ULK1 can regulate ROS production by promoting the Nrf-2/HO-1 pathway, affecting angiotensin II-induced cardiac hypertrophy progression.

In short, ULK1-mediated autophagy activation is beneficial to the heart in most cases, but excessive autophagy can aggravate the damage of the heart. In addition, ULK1 can also induce the production of ROS and induce cardiac hypertrophy. Therefore, due to the complex role of ULK1 in heart disease, there is still a long way to go to apply ULK1-related therapies to improve heart disease.

4.5. Other human diseases

4.5.1. Autophagic function

ULK1 also plays a unique role in some other organs, including the liver, bone, skin, etc. Associated diseases in these organs are generally accompanied by uncontrolled autophagy. For instance, SQSTM1/p62 activates NFE2L2/NRF2 and protects mouse liver against lipotoxicity through ULK1-mediated degradation of KEAP1 in autophagy. Moderate exercise and healthy diet can promote lipoprotein and improve non-alcoholic fatty liver disease and liver aging caused by a high-fat diet by activating AMPK/ULK1 and inhibiting Akt/mTOR/ULK1 pathway, respectively. Intriguingly, acupuncture, as traditional Chinese medicine therapy and alternative therapy, can prevent and treat diseases by regulating autophagy level. Electroacupuncture “Zusanli” can improve gastrointestinal motility disorder in rats with functional dyspepsia. Its mechanism may inhibit the excessive autophagy level of Cajal stromal cells by regulating the AMPK/ULK1 signal pathway. Unfortunately, the current research lacks unified conclusions and needs in-depth research.

In addition, ULK1/LC3 is inversely associated with renal fibrosis in diabetic patients, and dysregulation of autophagy leads to diabetic kidney disease, and activation of autophagy via p53/miR-214/ULK1 axis may alleviate diabetic kidney disease. Besides, by activating the p38MAPK/mTOR/ULK1Ser757 signaling pathway, sPLA2-IB downregulates autophagy through PLA2R and causes podocyte damage, which in turn impairs glomerular filtration. Furthermore, ULK1 expression is downregulated during osteoclast differentiation, and ULK1 may regulate osteoclasts differentiation through DOK3/Syk/JNK signaling in vitro, which is clinically relevant to osteoporosis. Therefore, activation of ULK1 is likely to be a promising therapeutic for osteoporosis. Indeed, osteoprotegerin could inhibit osteoclast bone resorption by Akt/mTOR/ULK1-induced autophagy. Notably, ULK1 has also been linked to skin diseases. In psoriasis, AMPK regulates autophagy through the ULK1/Atg7 signaling pathway and mitophagy through the PINK1/Parkin signaling pathway, thereby affecting the prognosis of a mouse model of psoriasis. Also, UVB irradiation can down-regulate the expression of ULK1 in human keratinocytes and reduce autophagy. SREBP-1c blocks ULK1 sulfuration-dependent activation by reducing the activity of cystathionine γ-lyase, resulting in decreased autophagic flux, which may be responsible for hepatic steatosis.

4.5.2. Non-autophagic function

Importantly, non-autophagic function of ULK1 also contributes to a lot in human disease. AMPK-activated ULK1 can help embryonic stem cells maintain self-renewal and maintain versatility. ULK1 can phosphorylate multiple sites of DSH in Wnt signaling pathway, which may affect Wnt signaling. Besides, ULK1 phosphorylates Ser843 in the binding region of mineralocorticoid receptor ligand in renal intercalated cells, to maintain water salt balance. In addition, the activation of mTORC1–ULK1–Atg13 pathway induced by rapamycin may alleviate the inflammatory response of rat model of chronic non-bacterial prostatitis. Furthermore, liver-specific ULK1 knockout can reduce the liver injury caused by acetaminophen. And p62 and ULK1 can be phosphorylated by PERK to protect the liver from lipotoxicity by activating the KEAP1–Nrf2 pathway.

Additionally, ULK1-regulated stearoyl-CoA desaturase 1 transcriptional regulation is associated with lipid metabolism in hepatocytes. Mechanistically, ULK1 increases transcription of stearoyl-CoA desaturase 1, reduces lipid droplet formation in the SFA model and alleviates lipotoxicity in hepatocytes. Further, ULK1 can also protects cells by controlling RIPK1-mediated cell death.

In general, ULK1 plays a complex and diverse role in a variety of human diseases. Due to the Jannus role of autophagy, the autophagy initiator ULK1 is inherently complicated, and its non-autophagy-related function also makes its role impossible to simply characterize. Therefore, the role of ULK1 needs to be further explored for ULK1-related therapeutics to have a promise for future potential therapeutic purpose.

5. Targeting ULK1 with pharmacological small-molecule compounds

More recently, some small-molecule compounds have been reported to affect disease progression and achieve therapeutic effects by modulating ULK1 (Fig. 7 and Tables 4–7). In this section, we focus on discussing some representative pharmacological small-molecule compounds that modulate the ULK1-mediated autophagy in different types of human diseases, analyzing their characteristics, advantages, and disadvantages, and looking forward to providing a clue on the development of ULK1-related drugs.

5.1. Small-molecule inhibitors

In 2015, based upon the crystallographic structure of ULK1 kinase domain, combined with kinase activity assay, the first ULK1 inhibitor compound was designed based on the structure of ULK1 by Lazarus, with ULK1 IC50 = 8 mmol/L. Unfortunately, it is not
a specific inhibitor for ULK1. Subsequently, a new scaffolding ULK1 inhibitor compound 3 was found to be a useful tool compound, whose discovery further deepened the understanding of the structure of ULK1 inhibitors, and it was higher selectivity to ULK1, while lower affinity compared to compound 6 (ULK1 IC_{50} = 120 ± 17 nmol/L, ULK2 IC_{50} = 360 ± 79 nmol/L)\(^{293}\). Another two ULK1 inhibitors, MRT67307 and MRT68921, showed excellent inhibitory effects on both ULK1 and ULK2, among which the IC_{50} of MRT67307 for ULK1 is 45 nmol/L, and the IC_{50} for ULK2 is 38 nmol/L, and MRT68921 exhibited better activity (IC_{50} for ULK1 = 2.9 nmol/L, IC_{50} for ULK2 = 1.1 nmol/L)\(^{298}\). Of note, MRT68921 displayed good anti-tumor activity \textit{in vitro} and \textit{in vivo}\(^{389}\). In addition, the tubulin polymerization inhibitor 5f was found to inhibit the enzymatic activity of various kinases, including ULK1. Although 5f has tumor-suppressive potential, its high cytotoxicity limits its use\(^{390}\).

Besides, the ULK1 small molecule inhibitor 3g with an indazole core was discovered through high-throughput in silico screening (HTS) followed by structure optimization. This compound was found to have a high selectivity to inhibit ULK1 (IC_{50} = 45 nmol/L) with outstanding stability \textit{in vivo} and good inhibition of CYP\(^{291}\). Interestingly, Egan and his colleagues also discovered another famous ULK1 kinase inhibitor SBI-0206965, with higher selectivity, with IC_{50} values of 108 nmol/L for ULK1 and 711 nmol/L for ULK2, which is the most used ULK1 inhibitor. In addition, SBI-0206965 could suppress ULK1-mediated phosphorylation events, inhibiting autophagy\(^{14}\). In addition, SBI-0206965 could down-regulate ULK1 and selectively inhibit ULK1-induced apoptosis in renal cell carcinoma cells\(^{223,292}\). Compared with SBI-0206965, SBP-7455 has increased binding affinity for ULK1/2 (IC_{50} for ULK1 = 13 ± 2 nmol/L, IC_{50} for ULK2 = 476 ± 21 nmol/L), potently inhibits ULK1/2 activity \textit{in vitro}, and reduces TNBC cell proliferation, and is orally bioavailable \textit{in vivo}. SBP-7455 inhibits autophagy for autophagy-dependent survival in TNBC cells even when starved and exhibits synergistic cytotoxicity against TNBC cells with PARP inhibitor olaparib\(^{293}\).

According to the characteristics of the interaction between ULK1 kinase and ULK1 inhibitors, the study of the features of chemical structures, as well as high-throughput virtual screening, 3s was found to be a good ULK1 inhibitor with 99.15% ULK1 kinase inhibitory activity at 10 nmol/L, higher than 97.56% of SBI-0206965. And 3s also exert a promising effect of anti-non-small cell lung cancer\(^{294}\). Additionally, ULK1 inhibitor XST-14 was discovered through structure-based virtual docking (IC_{50} value for ULK1 is 13.6 nmol/L), which acts as an anti-hepatoma drug by reducing autophagy and inducing apoptosis\(^{214}\). The above-mentioned ULK1-targeted inhibitors and their related mechanisms of action and applications are summarized in Table 4. It is worth noting that although a variety of ULK1 inhibitors have been discovered one after another with the modernization of medicinal chemistry, unfortunately, none of them can be called drugs in the true sense, nor have they entered clinical trials. Nevertheless, ULK1 inhibitors still have potential applications in human disease. Particularly, MRT68921, SBI-0206965, SBP-7455, and XST-14 all showed good anti-tumor effects in animal experiments, which also provided the possibility for their further development.

5.2. Small-molecule activators

Through TCGA analysis, TMA analysis, as well as in silico screening and chemical synthesis, LYN-1604 was discovered as a good candidate as a ULK1 activator and an excellent agent to treat TNBC by regulating autophagy. Notably, LYN-1604 is the first ULK1 activator with half effective concentration for activating ULK1 is 18.94 nmol/L, and showed good anti-TNBC activity \textit{in vitro} and \textit{in vivo}. Therefore, LYN-1604 may have therapeutic potential in future TNBC treatment\(^{172}\). Recently, another ULK1 activator BL-918 was found through structure-based drug design. BL-918 has an IC_{50} = 24.14 nmol/L for ULK1, and could rescue SH-SY5Y cells from MPP\(^{+}\) injury, and protects motor disorder and dopaminergic neuron loss induced by MPTP in mouse by activating ULK1-regulated autophagy (Table 4)\(^{392}\). Invigoratingly, the ULK1 activator to regulate autophagy holds promise for physiological and pharmacological perspectives for treating human diseases.

5.3. Small-molecule compounds modulating the ULK1-related pathways

5.3.1. Positive regulation of ULK1

As the most commonly canonical autophagic pathway, the AMPK/mTOR/ULK1 pathway affects multiple diseases by activating autophagy\(^{295}\). Correspondingly, several compounds could positive regulate ULK1 via this pathway. For instance, ginkgolide K\(^{296}\) promotes protective autophagy to promote astrocyte proliferation and migration after oxygen-glucose deprivation to treat ischemic stroke; Chikusetsu saponin Iva\(^{297}\) could relieve disease progression by increasing autophagic activity in isoproterenol-modelled mouse myocardial fibrosis. Melatonin\(^{297}\) mitigates vascular calcification through activation of autophagy. Importantly, AMPK/mTOR/ULK1 pathway also affects energy metabolism. In hypoxia, salidroside reduces pulmonary artery smooth muscle cells activity and its resistance to apoptosis by upregulating autophagy\(^{298}\). Alisil A 24-acetate inhibits oxidative stress and activates autophagy in mouse liver and LX-2 cells, which contribute to the treatment of nonalcoholic steatohepatitis\(^{299}\). Under high glucose conditions, berberine protects podocytes from damage by activating autophagy\(^{300}\). In addition, 10-hydroxycamptothecin\(^{301}\), FL-411\(^{302}\), gossypol acetate\(^{303}\) serve as anticancer agents by improving autophagic activity mediated by AMPK/mTOR/ULK1 pathway. Interestingly, in Drosophila, \(\beta\)-guanidinopropionic acid can contribute to prolonging lifespan by AMPK/Atg1 pathway\(^{304}\). Clozapine can stimulate the AMPK–ULK1–Beclin1 pathway by inducing the phosphorylation of ULK1 and Beclin1 and enhancing the levels of LC3-II and Atg5–Atg12, thus promoting autophagic flux to eliminate misfolded proteins in neural cells and degrade toxic substances\(^{305}\). Eicosapentaenoic acid could exert neuroprotective effects after MCAO by inducing AMPK/ULK1 signal and autophagy activation through PPAR\(\gamma\) signal\(^{306}\). Ezetimibe is an NPC1L1 inhibitor that activates the AMPK/ULK1/autophagy pathway by phosphorylating AMPK, ULK1, Beclin1, etc., reducing apoptosis of neurocyte, and is worthy of intensive study as a promising agent for treating ischemic stroke\(^{307}\). Additionally, compound Rg2 treats metabolic diseases by activating the AMPK/ULK1 autophagy pathway, such as the clearance of protein aggregates, high-fat diet-induced insulin resistance, etc\(^{308}\). Activation of the AMPK/ULK1 pathway by resveratrol could control pluripotency of embryonic stem cells in mice\(^{309}\). Compound A77 1726 inhibits S6K1 and promotes autophagy by increasing phosphorylation of AMPK\(^{310}\) and ULK1\(^{311}\), and enhances degradation of SOD1 protein for the treatment of ALS\(^{312}\). Kinsenoside activates AMPK–ULK1-dependent autophagy to alleviate alcoholic liver injury\(^{313}\). Activation of autophagy also...
plays a therapeutic role in cancer therapy regulated by AMPK/ULK1 signaling axis, such as baicalein against multiple cancers\textsuperscript{312,313}; narciclasine against TNBC\textsuperscript{314}; raloxifene\textsuperscript{315} against breast cancer; 3,3'-diindolylmethane against human PC\textsuperscript{316}. As for drug-resistant, temozolomide activates autophagic activity through ATM\textsuperscript{317}. Also, aescin induces ROS activation and promotes autophagy through activation of the ATM/AMPK/ULK1 pathway, which might promote cancer cell survival; thus, combined use with autophagy inhibitors may provide a therapeutic strategy for anti-tumor.\textsuperscript{318} Aspirin may induce autophagy by inhibiting mTOR signaling targets and increasing ULK1 and LC3A, thereby inhibiting angiogenesis in mouse liver cancer and sarcoma models\textsuperscript{319}. Additionally, the mTOR inhibitor AZD8055 inhibits phosphorylation of ULK1 at Ser757 to activate ULK1 activity and induces autophagic activity and downregulates p62 to antagonize chemotherapy-induced cell death\textsuperscript{320}. Polyphyllin VI promotes apoptosis and autophagic cell death in NSCLC through ROS-induced mTOR/ULK1 pathway and exerts potent anti-cancer proliferation in vitro and in vivo in its prototype form\textsuperscript{321,322}. In human breast cancer cells, flavonoids such as isorhamnetin, coriander, and acacetin could downregulate PI3K-γ-p110 and subsequently disrupt the PI3K/Akt/mTOR/p70S6K/ULK1 pathway, then inhibiting cancer cell proliferation by inducing cell cycle arrest at the G2/M phase of breast cancer cells, promoting apoptosis and autophagocytic cell death\textsuperscript{322}. In niclosamide-resistant cell lines, mTOR/ULK1 signaling, was blocked, and LC3B expression was increased, suggesting that autophagy may be a marker of niclosamide tumor resistance\textsuperscript{323}. Tizoxanide may enhance autophagic activity by blocking the Akt/mTOR/ULK1 signaling pathway in macrophages\textsuperscript{324}. In AD model mice, nitazoxanide, an anti-parasitic drug, could activate autophagic flux through PI3K/Akt/mTOR/ULK1 and NQO1/mTOR/ULK1 signaling pathways blockage to clear Aβ protein in the brain, which is beneficial to ameliorate learning and memory impairments\textsuperscript{228}. Further, licochalcone A (LicA), a novel chemotherapeutic agent, as a Bcl-2 inhibitor, which can induce autophagy in human HCC cells via ULK1/Atg13 and ROS pathways, and inhibition of LicA-induced ROS by the antioxidant NAC can enhance LicA-induced cells apoptosis, further inhibiting HCC\textsuperscript{325}. 4,4'-Dime-thoxychalcone can affect ULK1 to activate autophagy through JNK and MAPK kinases slowing skin aging\textsuperscript{326}. And 20-hydroxyecdysone can up-regulate Atg gene and induce autophagy in the silkworm fat body (Table 5)\textsuperscript{327}.
### Table 4  Small-molecule compounds that directly regulate ULK1 protein.

| Compd. | Structure | Activator/inhibitor | Target | Disease | ULK1 Activity | Biological Activity | Ref. |
|--------|-----------|---------------------|--------|---------|---------------|---------------------|------|
| Compound 6 | Inhibitor | ULK1 | / | IC_{50} = 8 nmol/L | Cell type:/ | Animal type:/ | 16 |
| XST-14 | Inhibitor | ULK1 | Hepatocellular carcinoma | ULK1 (IC_{50} = 26.6 nmol/L) | Cell type: HepG2 HCC cells, and L02 hepatocyte cells Animal type: nude mice. Effective dose 15 or 30 mg/kg | 214 |
| MRT68921 | Inhibitor | ULK1/2 | Cancer | ULK1 = 2.9 nmol/L, ULK2 = 1.1 nmol/L | Cell type: human cancer cell lines A549, H1299, NCI-H460, MNK45, U251, SW480, SW620, HCT116, Colo320 and HT-29, PC-3, U266 Animal type: mice. Effective dose: 20 mg/kg | 216,288,289 |
| SBI-0206965 | Inhibitor | ULK1/2 and AMPK | cc RCC | ULK1 (IC_{50} = 108 nmol/L); ULK2 (IC_{50} = 711 nmol/L) | Cell type: A498 cells, ACHN cells Animal type: C57BL/6NTac mice. Effective dose 10 μmol/L | 222,292 |
| Compound 3 | Inhibitor | ULK1/2 | / | ULK1 (IC_{50} = 120 nmol/L) ULK2 (IC_{50} = 360 nmol/L) | Cell type:/ | Animal type:/ | 287 |
| MRT67307 | Inhibitor | ULK1/2 | Cancer | ULK1 = 45 nmol/L, ULK2 = 38 nmol/L | Cell type: MEF cells | Animal type:/ | 288 |
| 5f | Inhibitor | AURKA, FLT3, GSK3A, MAP3K, MEK, RSK2, RSK4, PLK4, ULK1, and JAK1 | Lung cancer | / | Cell type: A549 NSCLC cells Animal type:/ | 290 |
| 3g | Inhibitor | ULK1 | / | ULK1 (IC_{50} = 45 nmol/L) | Cell type:/ | Animal type:/ | 291 |

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5.3.2. Negative regulation of ULK1

Some small molecular compounds may inhibit autophagy by inhibiting ULK1-related signaling pathways to treat diseases (Table 5). Vitexin inhibits autophagy dysfunction through mTOR/ULK1 pathway to alleviate MCAO-induced oxidative damage, apoptosis and inflammation, thereby improving cerebral ischemic stroke. Inhibition of ULK1 by WP1130 blocks autophagic flux through inhibition of deubiquitinase, enhancing ULK1 ubiquitination and ULK1 transfer to aggregates. Rocaglamide inhibits autophagy by inhibiting ULK1 at the protein translation level, a process that restores granzyme B levels in NK cells to increase NK cell sensitivity to NSCLC in cancer immunotherapy. Sunitinib-induced myocardial cytotoxicity is a severe side effect during treatment; and studies have found that the HSP90 inhibitor geldanamycin can reduce the side effect by degrading autophagy-related proteins such as ULK1 and inhibiting the autophagy pathway. Hyperoside inhibits AMPK/ULK1-mediated autophagy in a rat kidney injury model following D-gal treatment, thereby preventing age-related kidney injury. In addition, Dietary compound isoliquiritigenin reduces the total expression and phosphorylation of ULK1 in mammals and inhibits the expansion of TNBC cells through autophagy-mediated apoptosis.

5.4. Drug combination therapies

On account of the complex role of autophagy in diseases, drug combination therapy is increasingly recognized for better long-term prognosis and fewer side effects, especially when drug resistance occurs. The combination of autophagy inhibitor chloroquine and low concentration 5-FU can increase the ability of tumor cells to induce dendritic cell (DC) maturation by activating ULK1. Importantly, ULK1-mediated autophagy may be one of the reasons for the resistance in enzalutamide therapy in bladder cancer. Therefore, enzalutamide concurrent treatment with autophagy inhibitors (including 3-MA, BAF, and chloroquine) might be an emerging strategy to treat bladder cancer effectively. The combination of β-catenin inhibitor (FH535) and Akt inhibitor (AZD5363) enhances p53 expression and further induces lethal autophagy via regulation of the AMPK–mTOR–ULK1 pathway, exerting a more substantial effect on cell death of transformed hepatocytes, playing a treatment role in human hepatoma. Combination of ascorbic acid and menadione also exerts anticancer effects through the induction of cytotoxic autophagy in human glioblastoma through the AMPK/mTOR/ULK1 pathway. Curcumin pretreatment combined with 5-Fu enhanced the sensitivity of 5-Fu to colon cancer cells by inhibiting AMPK/ULK1-dependent autophagy (Table 6).

5.5. Other therapeutic strategies

Many protein drugs can also affect the expression of ULK1. Extracellular histones could promote autophagic and apoptotic activity through Sestrin2/AMPK/ULK1/mTOR and Akt/mTOR pathway to inhibit cultured human endothelial cells, which may be a novel target for therapeutic strategies in endothelial damage. Heavy metal scavenger can increase the phosphorylation of ULK1 and inhibit the phosphorylation of eNOS, reducing the abnormal myocardial contraction caused by deep hypothermia by attenuation of cardiac autophagy. A new bursal heptapeptide (BP7) stimulates autophagy and phosphorylation of AMPK/ULK1 pathway and regulates the expression of Bcl-2 protein in WEHI-
Table 5  Small-molecule compounds that regulate the ULK1 pathways.

| Compd.             | Structure | Activation/inhibition | Target                               | Disease                | Biological activity                                                                 | Ref.  |
|--------------------|-----------|-----------------------|--------------------------------------|------------------------|-------------------------------------------------------------------------------------|-------|
| Nitazoxanide       | ![Structure](image) | Activation           | PI3K/AKT/mTOR/ULK1 and NQO1/mTOR/ULK1 pathways | AD                     | Cell type: BV2, SH-sy5y. Effective dose: 20 μmol/L. Animal type: APP/PS1 transgenic mice. Effective dose: 90 mg/kg | 228   |
| Chikusetsu saponin Iva | ![Structure](image) | Activation           | AMPK/mTOR/ULK1 pathway               | Cardiac fibrosis       | Cell type: / Animal type: BALB/c mice. 5 or 15 mg/kg                                  | 265   |
| Ginkgolide K       | ![Structure](image) | Activation           | AMPK/mTOR/ULK1 pathway               | Ischemic stroke        | Cell type: astrocyte. Animal type: /                                                 | 296   |
| Melatonin          | ![Structure](image) | Activation           | AMPK/mTOR/ULK1 pathway               | Vascular calcification | Cell type: vascular smooth muscle cells. Animal type: /                                | 297   |
| Salidroside        | ![Structure](image) | Activation           | AMPK/mTOR/ULK1 pathway               | Pulmonary hypertension | Cell type: pulmonary arterial smooth muscle cells (PASMCs). Animal type: /            | 298   |
| Alisol A 24-acetate | ![Structure](image) | Activation           | AMPK/mTOR/ULK1 pathway               | Nonalcoholic steatohepatitis | Cell type: LX-2 human liver astrocyte cells. Animal type: C57BL/6 mice. Effective dose: 15, 30 and 60 mg/kg | 299   |
| Berberine          | ![Structure](image) | Activation           | AMPK/mTOR/ULK1 pathway               | /                      | Cell type: MPC5 podocytes. Animal type: /                                             | 300   |
| FL-411             | ![Structure](image) | Activation           | AMPK/mTOR/ULK1 pathway               | Bladder cancer         | Cell type: T24 and 5637 human bladder cancer cells. Animal type: /                    | 301   |
| Gossypol acetate   | ![Structure](image) | Activation           | AMPK/mTOR/ULK1 pathway               | Colon cancer, lung cancer | Cell type: HCT116 cells and A549 cells. The best dose: 10 μmol/L                      | 302   |

(continued on next page)
| Compd. Structure | Activation/ inhibition | Target | Disease | Biological activity | Ref. |
|------------------|------------------------|--------|---------|---------------------|-----|
| **β-Guanidinopropionic acid** | Activation | AMPK/mTOR/ULK1 pathway | / | Cell type: *Drosophila* melanogaster. the effective dose to extend the lifespan of *Drosophila* melanogaster: ≥900 mmol/L | 304 |
| Clozapine | Activation | AMPK/ULK1 pathway | Psychotic disorders | Cell type: | 305 |
| Eicosapentaenoic acid | Activation | AMPK/ULK1 pathway | Evaporative dry eye disease | Cell type: human meibomian gland epithelial cells | 306 |
| Ezetimibe | Activation | AMPK/ULK1 pathway | Middle cerebral artery occlusion | Cell type: Male Sprague-Dawley rats. Effective dose: 10 mg/kg | 307 |
| Rg2 | Activation | AMPK/ULK1 pathway | AD | Cell type: HeLa human cervical carcinoma cell, Neuro2A Mouse brain neurona cell, PC12 pheochromocytoma cell. The dose used in Hela cell: 50 mmol/L | 308 |
| Resveratrol | Activation | AMPK/ULK1 pathway | / | Cell type: mouse embryonic stem cells, the dose used in mouse embryonic stem cells: 10 μmol/L | 309 |
| A77 1726 | Activation | AMPK/ULK1 pathway | ALS | Cell type: NSC34 mouse neuron cells. The effective dose used in NSC34 cells: 200 μmol/L | 310 |
| Kinsenoside | Activation | AMPK/ULK1 pathway | Alcoholic fatty liver | Cell type: AML12 cells. The best dose: 40 μmol/L | 311 |
| Baicalein | Activation | AMPK/ULK1 pathway | Human prostate and breast cancer | Cell type: PC-3 human prostate cells, DU145 human prostate cells and MDA-MB-231 breast cancer cells. The effective dose used in DU145 cells: 2.5 μg/mL | 312 |
| Narcilasine | Activation | AMPK/ULK1 pathway | TNBC | Cell type: HCC-1937 and MDA-MB-231 breast cancer cells. the dose used in the HCC1937 cells: 20 or 50 mmol/L | 314 |
| Compound                  | Activation Pathway | Cancer Type      | Cell Type                  | Animal Type                          |
|--------------------------|--------------------|------------------|----------------------------|--------------------------------------|
| Raloxifene               | Activation AMPK/ULK1 pathway | Breast cancer | MCF-7 human breast cancer cells | Male Kunming mice                    |
| 3,3'-Diindolylmethane    | Activation AMPK/ULK1 pathway | Human prostate cancer | LNCaP and C42B human prostate cancer cells | Male Kunming mice                    |
| Temozolomide             | Activation ATM/AMPK/ULK1 pathway | Glioma          | U87MG and U251 human malignant glioma cells | Male Kunming mice                    |
| Aescin                   | Activation ATM/AMPK/ULK1 pathway | HCC and colon carcinoma | HepG2 and HCT116 human colon carcinoma cells | Male Kunming mice                    |
| Aspirin                  | Activation mTOR/ULK1 pathway | Hepatocarcinoma and sarcoma | H22 hepatocarcinoma and S180 sarcoma cells | Male Kunming mice                    |
| AZD8055                  | Activation mTOR/ULK1 pathway | /               | HT-29, DLD-1 human colon carcinoma cells | Male Kunming mice                    |
| Polyphyllin VI           | Activation AMPK/mTOR/ULK1 pathway | Non-small cell lung cancer | A549 NSCLC cells, and H1299 NSCLC cells | Male Kunming mice, and BALB/c nude mice |
| Isorhamnetin (IH)        | Activation PI3K/AKT/mTOR/p70S6K/ULK1 pathway | TNBC            | MDA-MB-231 breast cancer cells | Male Kunming mice                    |
| Genkwanin (GN)           | Activation PI3K/AKT/mTOR/p70S6K/ULK1 pathway | TNBC            | MDA-MB-231 breast cancer cells | Male Kunming mice                    |
| Acacetin                 | Inhibition PI3K/AKT/mTOR/p70S6K/ULK1 pathway | TNBC            | MDA-MB-231 breast cancer cells | Male Kunming mice                    |

(continued on next page)
| Compd.       | Structure | Activation/inhibition | Target                          | Disease                   | Biological activity                                                                 | Ref. |
|-------------|-----------|-----------------------|---------------------------------|---------------------------|--------------------------------------------------------------------------------------|------|
| Niclosamide | ![Structure](image1.png) | Inhibition            | mTOR/ULK1 pathway                | Ischemic stroke           | Cell type: HEK293T human embryonic kidney cells. HCT116 colon cancer cell (IC\textsubscript{50} = 0.31 μmol/L) Animal type:/ | 323  |
| Tizoxanide  | ![Structure](image2.png) | Activation            | PI3K/Akt/mTOR/ULK1 pathway       | Virus                     | Cell type: HepG2 HCC cells. Animal type:/                                              | 324  |
| LicA        | ![Structure](image3.png) | Activation            | ULK1/Atg13 and ROS pathway       | HCC                       | Cell type: HuH7 and HepG2 HCCs cells. The best dose: 50 μmol/L Animal type:/           | 325  |
| 4,4'-Dimethoxychalcone | ![Structure](image4.png) | Activation            | p38 and JNK pathway              | Aging                     | Cell type: HaCaT cells. The best dose: 20 μmol/L Animal type: mice. Effective dose: 50, 100 mg/kg Animal type: the silkworms. Effective dose: 5 μg/larva | 326  |
| 20-Hydroxyecdysonone | ![Structure](image5.png) | Activation            | Atg genes                        | /                         | Cell type: silkworm. The dose used in HEK 293 cells: 1 μmol/L Animal type: the silkworms. Effective dose: 5 μg/larva | 327  |
| Vitexin     | ![Structure](image6.png) | Inhibition            | mTOR/ULK1 pathway                | Cerebral ischemic stroke  | Cell type:/ Animal type: MACO model SD rats. Effective dose: 2 mg/kg                  | 328  |
| WP1130      | ![Structure](image7.png) | Inhibition            | ULK1, USP9X                      | Cervical cancer and osteosarcoma | Cell type: HeLa cervical cancer cells U20S human osteosarcoma cells Animal type:/ | 329  |
| Geldanamycin| ![Structure](image8.png) | Inhibition            | Atg7, Beclin-1, ULK1             | The cytotoxicity of sunitinib in cardiomyocytes | Cell type: NH9c2 rat myocardial cells. Animal type:/ | 331  |
| Hyperoside  | ![Structure](image9.png) | Inhibition            | AMPK/ULK1 pathway                | Renal aging               | Cell type: NRK-52E rat kidney cells Animal type:/                                     | 332  |
| Isoliquiritigenin | ![Structure](image10.png) | Inhibition            | AMPK/mTOR/ULK1 pathway           | TNBC                      | Cell type: MDA-MB-231 breast cancer cell Animal type: female Nude-Foxn1 mice. Effective dose: 2.5 or 5 mg/kg | 333  |
### Table 6  Drug combination therapy.

| Compd. 1 | Structure 1 | Compd. 2 | Structure 2 | Activation/Inhibition | Target | Cell type | Disease | Ref. |
|----------|-------------|----------|-------------|------------------------|--------|-----------|---------|------|
| Curcumin | ![Structure](image1) | 5-FU | ![Structure](image2) | Inhibition | AMPK/ULK1 autophagy | HCT116 and HT29 colon cancer cells | Colon cancer | 208 |
| Chloroquine | ![Structure](image3) | 5-FU | ![Structure](image4) | Activation | ULK1 | HCT-116 colon cancer cells | Colon cancer | 334 |
| Enzalutamide | ![Structure](image5) | 3-MA | ![Structure](image6) | Inhibition | AMPK/mTOR/ULK1 pathway | J82, T24, and UMUC3 human bladder cancer cells | Bladder cancer | 335 |
| Enzalutamide | ![Structure](image7) | BAF | ![Structure](image8) | Inhibition | AMPK/mTOR/ULK1 pathway | J82, T24, and UMUC3 human bladder cancer cells | Bladder cancer | 335 |
| Enzalutamide | ![Structure](image9) | CQ | ![Structure](image10) | Inhibition | AMPK/mTOR/ULK1 pathway | J82, T24, and UMUC3 human bladder cancer cells | Bladder cancer | 335 |
| AZD5363 | ![Structure](image11) | FH535 | ![Structure](image12) | Activation | AMPK/mTOR/ULK1 pathway | HepG2 HCC cells and Hep3B cells | Hepatocellular carcinoma | 336 |
| Ascorbic acid | ![Structure](image13) | Menadione | ![Structure](image14) | Activation | AMPK/mTORC1/ULK1 pathway | Glioblastoma | Glioblastoma Cells | 337 |

### Table 7  Other therapeutic strategies.

| Drug | Activation/Inhibition | Target | Cell type | Disease | Ref. |
|------|-----------------------|--------|-----------|---------|------|
| Heavy metal scavenger | Activation | Phosphorylation of ULK1 | / | Myocardial contractile anomalies | 269 |
| Extracellular histones | Activation | Sestrin2/AMPK/ULK1–mTOR and AKT/mTOR | Human endothelial cell | / | 338 |
| The bursa of Fabricius (BP7) | Activation | AMPK–ULK1 phosphorylation | WEHI-231 mouse B lymphocytes cells | / | 339 |
| Adiponectin | Activation | LC3, Beclin1, ULK1 | L6 skeletal muscle cells | Diabetes | 339 |
ULK1 is a key regulator in the initiation of mammalian autophagy, and its function is conserved in all eukaryotes. Accordingly, ULK1 has a potential to fight human diseases as a promising therapeutic target. With the in-depth studies of ULK1, whether it is the canonical autophagy reaction played by ULK1 by autophagy–lysosomal pathway, or its independent of autophagy role in stabilizing oxidative stress and endoplasmic reticulum stress, reducing lipid, promoting erythrocyte maturation, as well as regulating innate immune response, shows that ULK1 plays an irreplaceable role in normal physiological activities and regulating pathological processes.

Moreover, autophagy may be beneficial or detrimental depending on the type or stage of one disease. For example, ULK1 plays the Janus role in different types and stages of human cancers. According to the specific development stages and types of a particular tumor, developing small-molecule drugs targeting ULK1 to impede the tumor progression is a worthy researching direction. For neurodegenerative diseases, ULK1 mainly plays a protective role by activating autophagy. But in a few neurodegenerative diseases such as Chorea-acanthocytosis, ULK1 can play a pathogenic role. The specific role of ULK1 in various nervous system diseases still needs to be explored by further deepening study. In immune diseases, whether ULK1-mediated autophagy acts to fight viral invasion or promote viral replication depends on the type of virus. ULK1-mediated moderate autophagy activation is beneficial to the heart, but excessive autophagy can aggravate the process of heart disease. The precise correction of the uncontrolled ULK1 is appropriate. In addition, there are few studies on the unique role of ULK1 in tissues and organs, such as skin, kidneys, bone, and liver. Thus, whether ULK1 plays a therapeutic role or a toxic reaction is also worthy of further investigations. Interestingly, ULK1 is explicitly expressed in some organs or lesions, facilitating the pathological process via non-autophagy pathways. For example, the anti-migration effect of ULK1 phosphorylation of Exo70 in tumors is not achieved through the autophagy pathway. Due to its structural characteristics, ULK1 plays a key role in regulating neuronal development. In addition, ULK1 promotion can directly affect the antiviral effect of IFN-γ. ULK1 in the adult hearts treats cardiomyopathy and heart failure through a non-autophagic pathway. As mentioned above, the non-autophagic role of ULK1 is also crucial in human diseases, beyond its inherent autophagy.

Since ULK1 is destined to have multifaceted roles in diverse human diseases, developing therapeutic approaches that integrate multiple pathways may be a successful therapeutic strategy. In some cases, the improvement of ULK1 for disease is based on its autophagy regulation function. Under this circumstances, excessive inhibition/activation of ULK1 may have unpredictable pathogenic effects on the other organ and tissue by affecting autophagy. Thus, specific regulation of target organ ULK1 may address this issue, and correction of abnormal modifications of disease-specific ULK1 may also avoid the damage to other tissues. In addition, more studies need to elucidate the precise function of ULK1-mediated autophagy in individual disease types and pathological processes before ULK1-targeted therapy can be considered. In other cases, ULK1 may ameliorate the disease through its non-autophagy function. At this time, the regulation of ULK1 on autophagy may bring risks to the treatment of the disease. In this case, a combination of multiple treatment regimens may be more appropriate (e.g., combined with autophagy inhibitors to correct autophagy levels under certain circumstances). Additionally, considering that the inhibition/activation of ULK1 appears to be limited by differences in ULK1 expression levels and basal values of autophagy in different types of organs/lesions, therapeutic strategies targeting ULK1 may have significant limitations. Thus, the combination of mechanism-based ULK1 inhibitors/activators with chemotherapeutics or targeted drugs may be more practical. Notably, the development of specific novel ULK1-targeting small-molecule drugs (targeting specific sites, specific modifications, specific PPI networks, etc.) may provide some new possibilities for potential applications of targeting ULK1 in human disease.

Unfortunately, the current research on inhibitors and activators targeting ULK1 to treat diseases is in the preclinical stage since the discovery of ULK1 inhibitors/activators is still in its infancy. It’s exciting that another autophagy-related target, mTOR, is more mature in clinical research. For example, the mTOR inhibitor sirolimus combined with metformin is already in Phase 1 trials in advanced solid tumors. (NCT0145559) Notably, autophagy contributes a lot in this trial. Compared with mTOR, ULK1 has obvious autophagy induction ability, and the clinical application of drugs targeting ULK1 is extremely promising. There’s optimism that some small molecular compounds targeting ULK1 exhibited good activity in vitro. For example, the ULK1 inhibitor SBI-026965 and the ULK1 activator LYN-1604 showed excellent anticancer effects in nude mice, and the ULK1 inhibitor XST-14 displayed good pharmacokinetics in mice. As mentioned above, the clinical performance of ULK1 inhibitors/activators is worth for further expectations.

Currently, multiple issues and challenges have remained in translating knowledge on ULK1 into the clinic. First, the applications of small-molecule compounds targeting ULK1 should be attributed to a comprehensive understanding of its mechanisms of action in vitro and in vivo, but only part of it has been well elucidated. For example, the crystal structures of ULK1 and ULK1 complex have not been fully resolved, and increasing research in this area will help the discovery of small-molecule compounds that specifically target ULK1 and be further used for potential treatment. Further, potential toxic and side effects are the critical issue hindering the clinical administration of drugs. Notably, in the era of precision medicine, whether the non-autophagic function of ULK1 could guide the stratification of patients in clinical trials by determining ULK1 as a biomarker, which will facilitate the development of gene drugs, RNAi drugs, and antibody drugs targeting ULK1. Additionally, the functions of ULK1 in non-autophagy can contribute to predicting the most likely outcome for the patients who would benefit from ULK1 inhibitors/activators in a clinical setting. Therefore, elucidating the role of the non-autophagic function of ULK1 in various diseases is helpful for the development of drugs targeting ULK1. In general, addressing the above questions requires an in-depth understanding of the molecular mechanisms of ULK1 in some regulatory events, which may provide the possibility for the clinical and applications of ULK1-targeted therapy. The excitement that there are some novel methods to discover further the relations of the biological functions of ULK1 and human diseases and to find novel small
molecular compounds for activating or inhibiting ULK1, such as CRISPR/Cas9, PRATACs, and artificial intelligence/machine learning. Pioneer studies have shown that disruption of the ULK1 gene can be easily knocked out or introduced by CRISPR/Cas9-induced dual fluorescent reporter genes. PROTACs could effectively inactivate ULK1 protein function via compound-mediated proteolysis. Artificial intelligence/machine learning is used to estimate autophagy protein levels. Autophagic proteins including ATG1, ATG5, and LC3B can be quantified by software-based integrated optical density to characterize alters in basal autophagy levels of patients.

In summary, these inspiring findings demonstrate the molecular structure and biological functions of ULK1, elucidating its regulatory role in both autophagy and non-autophagy, which may ultimately provide a new clue on our understanding of the autophagic initiator ULK1. Moreover, we focus on discussing a comprehensive exploration of the complicated relationship between ULK1 and human diseases, establishing a specific theoretical basis for ULK1 for potential therapeutic purposes. The discovery of small-molecule drugs targeting ULK1 will become one of the dominant therapeutic strategies for fighting human diseases. More importantly, some new emerging technologies have still been expected to help discover the first-in-class drugs targeting ULK1; and discovery of more candidate small-molecule drugs would contribute to driving ULK1-targed therapy to the future clinic.

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Author contributions

Bo Liu and Jin Zhang conceived the project and supervised the project. Ling Zou, Minru Liao, Yongqi Zhen, and Shiou Zhu summed up the literature and drafted the manuscript. Xiya Chen was involved in chemical structure drawing. Ling Zou and Jin Zhang proofread the structure and figures. Bo Liu, Yue Hao, and Jin Zhang revised the manuscript. All authors approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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