Acta Pharmaceutica Sinica B 2023;13(12):4688–4714

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

Targeting autophagy and beyond: Deconvoluting the complexity of Beclin-1 from biological function to cancer therapy

Jing Ye[a,†], Jin Zhang[a,†], Yanghui Zhu[a], Lian Wang[a,b], Xian Jiang[a], Bo Liu[a,*], Gu He[a,b,*]

[a]*Department of Dermatology, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, China*
[b]*Laboratory of Dermatology, Clinical Institute of Inflammation and Immunology (CIII), Frontiers Science Center for Disease Related Molecular Network, Chengdu 610041, China*

Received 3 May 2023; received in revised form 5 July 2023; accepted 2 August 2023

KEY WORDS

Beclin-1;
PI3KC3 complex;
Autophagy;
Non-autophagy;
Cancer therapy

Abstract Beclin-1 is the firstly-identified mammalian protein of the autophagy machinery, which functions as a molecular scaffold for the assembly of PI3KC3 (class III phosphatidylinositol 3 kinase) complex, thus controlling autophagy induction and other cellular trafficking events. Notably, there is mounting evidence establishing the implications of Beclin-1 in diverse tumorigenesis processes, including tumor suppression and progression as well as resistance to cancer therapeutics and CSC (cancer stem-like cell) maintenance. More importantly, Beclin-1 has been confirmed as a potential target for the treatment of multiple cancers. In this review, we provide a comprehensive survey of the structure, functions, and regulations of Beclin-1, and we discuss recent advances in understanding the controversial roles of Beclin-1 in oncology. Moreover, we focus on summarizing the targeted Beclin-1-regulating strategies in cancer therapy, providing novel insights into a promising strategy for regulating Beclin-1 to improve cancer therapeutics in the future.

*Corresponding authors. Tel.: +86 28 85503817; fax: +86 28 85164063.
E-mail addresses: liubo2400@163.com (Bo Liu), hegu@scu.edu.cn (Gu He).
†These authors made equal contributions to this work.

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2023.08.008

1. Introduction

As a cellular self-degradation mechanism, autophagy is extremely conserved in eukaryotes. In the autophagy process, cellular elements (*e.g.*, nucleic acids, proteins, lipids, and organelles) can be decomposed and recycled to deal with nutritional deficits or address cell stress for maintaining intracellular homeostasis. Autophagy includes three subclassifications: macroautophagy (henceforth referred to as autophagy), microautophagy, and chaperone-mediated autophagy. Autophagy as the major regulated degradation mechanism, is the best characterized process. It has been reported that autophagy plays a Janus role in cancer progress[1,2]. Generally, autophagy exhibits a tumor-suppressive effect in physiological conditions *via* degrading damaged cellular elements and eliminating abnormal cells to prevent the initiation and progression of malignancies[2−4]. However, autophagy can also have a cytoprotective role in cancer cells *via* providing the nutritional demands for cancer cells survival and protecting cells from drug-induced damage, leading to the development of malignancies and the resistance to therapeutics[5−8].

Autophagy machinery is controlled by ATG (autophagy-related) genes and proteins[9,10]. The key ATG proteins are functionally categorized into six groups: the ULK1 (Unc-51 like kinase 1, Atg1 in yeast) complex (containing ULK1, ATG101, FIP200, and ATG13), the PI3KC3 (class III phosphatidylinositol 3 kinase) complex, the ATG2−WIPI (WD repeat protein interacting with phosphoinositides, Atg18 in yeast) complex, the ATG12−ATG5−ATG16L1 conjugation system, the LC3 (microtubule-associated protein light chain 3, Atg8 in yeast) protein lipidation system, and ATG9-containing vesicles[11−14]. In 1998, yeast Apg6, also known as Atg6, was found to regulate autophagy by forming a protein complex[15]. Beclin-1 protein was initially cloned and identified as a novel Bcl-2-interacting protein in the same year by Beth Levine's laboratory[16]. In 1999, the study of Levine's group[17] further demonstrated that Beclin-1 was the mammalian ortholog of the yeast Atg6 that was an essential component for autophagy initiation. Similarly, in mammals, Beclin-1 was found to be a part of PI3KC3-C1 (PI3KC3 complex I, containing Beclin-1, VPS34, VPS15, and ATG14) and PI3KC3-C2 (PI3KC3 complex II, containing Beclin-1, VPS34, VPS15, and UVRAG), which mediate the formation and maturation of autophagosome and endocytic trafficking[18−22]. Furthermore, extensive research by numerous labs revealed that many transcription factors, epigenetic modulators, RNAs, kinases, and interacting proteins involves in regulating the function and activity of Beclin-1, which is help to understand the role of Beclin-1 in physiological and pathological processes, especially in tumorigenesis. Research into the relationship between Beclin-1 and cancer can be traced back to 1999, Levine's group[17] reported that Beclin-1 could contribute to inhibiting the development or progression of human malignancies, and allelic loss of *BECN1* can be observed in 75% of ovarian, 50% of breast, and 40% prostate cancers[23−25]. Since then, Beclin-1 has been identified as a haplo-insufficient tumor suppressor, and the low expression level of Beclin-1 has been linked with carcinogenesis and poor prognosis of patients in diverse cancers (*e.g.*, ovarian, gastric, breast, prostate, cervical, brain, and liver cancers, as well as oral/hypopharyngeal squamous cell carcinoma, melanoma, lymphoma, chondrosarcoma, and cholangiocarcinoma)[26−40]. Nevertheless, given the dual effects of autophagy, Beclin-1 as an autophagy activator has been recently discovered that plays a controversial role in promoting oncogenesis, inducing resistance to cancer therapeutics, and contributing to CSC (cancer stem-like cell) maintenance[41−44]. In 2013, an autophagy-inducing peptide derived from a region of Beclin-1, known as Tat-Beclin-1, was first developed for the treatment of virus-infected diseases[45]. In 2018, Tat-Beclin-1 was used to treat HER2 (human epidermal growth factor receptor 2)-positive human breast cancer xenografts in mice models, which showed a potent antitumor efficacy comparable to the clinical drug lapatinib (the HER2 inhibitor)[46]. Therefore, targeting Beclin-1 has emerged as a potential strategy for cancer therapy, and several targeted peptides, small molecules, and other products of biomedical engineering have been developed for the treatment of cancer (Fig. 1).

Here, we introduce the structure and functions of Beclin-1 in autophagic and non-autophagic processes. Besides, we further review its transcriptional and post-transcriptional regulations as well as post-translational modifications. In addition, we discuss recent advances in understanding the role of Beclin-1 in cancer, based on its autophagic and non-autophagic functions. Moreover, we focus on summarizing the development of targeted Beclin-1-regulating strategies in cancer therapy, highlighting the promising prospects of targeting Beclin-1 for the future cancer therapeutics.

2. Structure and function of Beclin-1

2.1. *Structure of Beclin-1*

Human Beclin-1 encoded by *BECN1* gene is a 450-amino-acid protein of ~60 kDa molecular weight (Fig. 2A), containing several structural domains: a B cell lymphoma 2 (Bcl-2)-homology-3 (BH3) motif (residues 105−130), a flexible helical domain (F, residues 141−171), a coiled-coil domain (CCD, residues 175−264), and an evolutionarily conserved domain (ECD, residues 248−337) together with a β/α-repeated, autophagy-related (BARA, residues 265−450) domain[19,47−49].

2.2. *Beclin-1 interactomes*

Many Beclin-1 interactomes can modulate the function of Beclin-1 (Fig. 2B). With ~25% sequence homology to myosin-like proteins, the CCD of Beclin-1 is essential for its scaffolding role for forming PI3KC3 complexes[16]. ATG14L and UVRAG can competitively bind to Beclin-1 at CCD, and their mutually exclusive interaction with Beclin-1 activates the VPS34 activity, resulting in the formation of PI3KC3-C1 or PI3KC3-C2, respectively[21,50,51]. VPS34 can interact with Beclin-1 at ECD, which is required for autophagy activation as well as tumor suppression[19]. Anti-apoptotic Bcl-2-like proteins [*e.g.*, Bcl-2, Bcl-X_L, Bcl-B, and Mcl-1 (myeloid cell leukemia-1)] can bind to Beclin-1 at the BH3 motif and suppress the interaction of Beclin-1 with ATG14L/UVRAG, leading to the inhibitory effect against autophagy induction[52−54]. Rubicon protein containing a RUN domain and a cysteine-rich domain can negatively regulate autophagy *via* interacting with Beclin-1 at the BARA domain to reduce VPS34 activity[55]. Ambra1 (activating molecule in Beclin-1-regulated autophagy protein-1) interacts with Beclin-1 at BH3 and CCD[56,57]. As a pro-autophagic effector, Ambra1 can competitively displace Bcl-2/Bcl-X_L, thus stabilizing the Beclin-1−VPS34 interaction[58,59]. Bif-1 (Bax-interacting factor-1) is also a pro-autophagic molecule, which is capable of interacting with Beclin-1 at CCD through UVRAG to modulate autophagy and tumorigenesis[60]. Additionally, HMGB1 (high mobility group box 1)

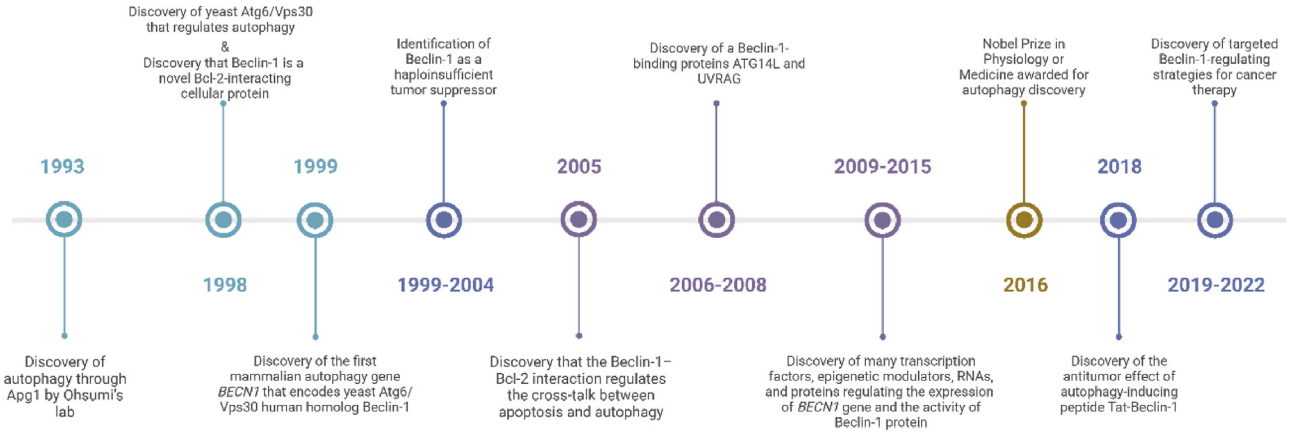


Figure 1 The mile-stone progress of the autophagy protein Beclin-1 from biological functions to cancer therapy.

can enhance autophagic flux by directly binding to Beclin-1 at CCD and causing the disassociation of the Beclin-1−Bcl-2 interaction[61,62]. A 26 kD GTPase DIRAS3 (a distinct subgroup of the RAS family member 3) serves imprinted tumor suppressor and interacts with the N-terminal region of Beclin-1, which can inhibit the Beclin-1 homodimerization and the Beclin-1−Bcl-2 interaction and promote the Beclin-1−ATG14L interaction to induce autophagy[63]. As a member of the NLR (nucleotide-binding and oligomerization

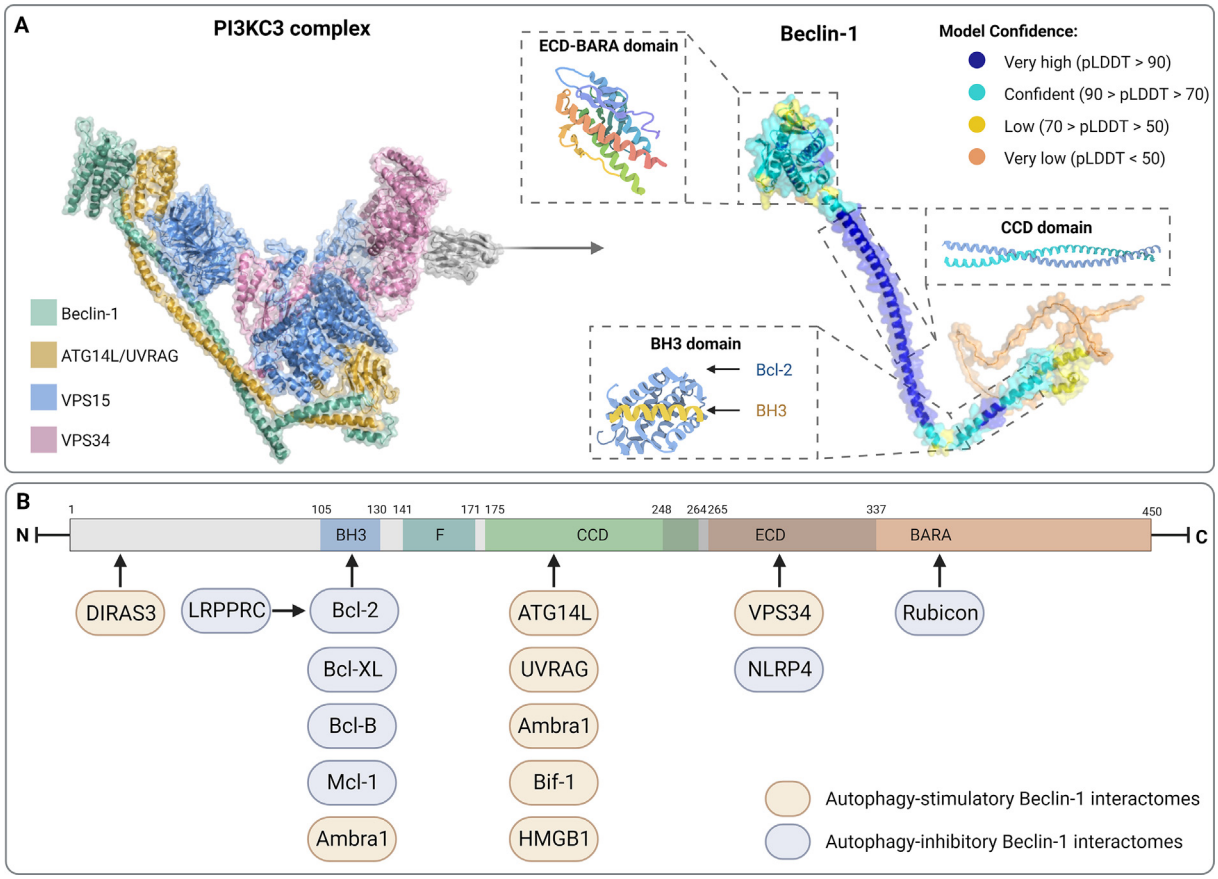


Figure 2 The molecular structure of Beclin-1 and its interactomes. (A) The molecular structure of PI3KC3 complex and Beclin-1. Crystal structure of PI3KC3 complex from yeast (PDB ID: 5DFZ) resolved to 4.40 Å by X-ray diffraction: VPS30 (Beclin-1 homolog, light green), Atg14/VPS38 (ATG14L/UVRAG homolog, Blue), VPS15 (cyan), and VPS34 (green). The three-dimensional structure of Beclin-1 is predicted by AlphaFold (https://alphafold.ebi.ac.uk/entry/Q53F78), which consists of several structural domains, including a BH3 motif (PDB ID: 5VAU), a CCD (PDB ID: 3Q8T), and an ECD-BARA domain (PDB ID: 3VP7). AlphaFold produces a per-residue confidence score (pLDDT) between 0 and 100, and residues with different pLDDTs are shown in different colors. (B) Schematic of the major Beclin-1 protein domains and their respective interactomes.

domain-like receptor) family members, NLRP4 shows a high affinity to the ECD of Beclin-1 and functions as a negative effector of the autophagic process[64]. LRPPRC (leucine-rich pentatricopeptide repeat-containing protein) is a mitochondrion-associated protein that inhibits autophagy by forming a ternary complex with Beclin-1 and Bcl-2 to stabilize the Beclin-1−Bcl-2 interaction[65].

2.3. *Functions of Beclin-1*

As a PI3KC3 complex member, Beclin-1 can function in autophagy and cellular trafficking processes. Currently, numerous non-autophagic functions of Beclin-1 in apoptosis, ferroptosis, necroptosis, DNA damage response, tumor metastasis, angiogenesis, and immune regulation have been revealed.

2.2.1. *Functions as a PI3KC3 complex member*

2.2.1.1. PI3KC3-C1. Structurally, the tetrameric PI3KC3-C1 can directly contact with membrane structures, and the aromatic finger (residues 359−361) in the BARA domain of Beclin-1 has a vital role in membrane association[66]. The PI3KC3-C1 contains two-armed V-shaped tips for membrane interaction: the N-terminal myristoylation site of VPS15 and the kinase domain of VPS34 together with the BARA domain of Beclin-1 and the Barkor autophagosome targeting sequence domain of ATG14L[67,68]. This domain of ATG14L is capable of sensing membrane curvature and preferentially promoting PI3KC3-C1 contact with the high-curvature membrane, which confers a critical role for PI3KC3-C1 in the nucleation and elongation of phagophore[69−71]. During autophagosome biogenesis, Beclin-1 as a core subunit of PI3KC3-C1 plays a role in the formation of autophagosomes[72,73]. The autophagy-inducing signals (*e.g.*, starvation and cellular stress) can activate the ULK1 complex[74,75], which then recruits the PI3KC3-C1 to the initiation site for autophagosome formation and phosphorylates components of the PI3KC3-C1 (including Beclin-1) to trigger the nucleation of phagophore[76−78]. ATG9-containing vesicles have been suggested to supply membranes for autophagosome precursor formation[11,12]. The ATG14L mediates the lipid specificity to target membranes[79], thus allowing that PI3KC3-C1 can recognize its substrate phosphatidylinositol and promote the production of PI3P (phosphatidylinositol 3-phosphate)[80]. The nucleation of the phagophore takes place within the PI3P-enriched subdomain of ER (endoplasmic reticulum), named omegasome[80]. The enrichment of PI3P contributes to recruiting ATG2−WIPI complex to omegasomes[81,82]. WIPI recruits the ATG12−ATG5−ATG16L1 complex to conjugate LC3 proteins with PE (phosphatidylethanolamine)[83−85], meaning that the phagophore is nucleated. Lipidated LC3 (LC3-PE) proteins not only function as a scaffold for core autophagy proteins (*e.g.*, the ULK1 complex and PI3KC3-C1) but also provide recognition sites for autophagy cargo receptors (*e.g.*, SQSTM-1/p62), which are essential for later autophagy process (Fig. 3A)[86−88]. Various post-translational modifications of Beclin-1, which are discussed in the next section, can mediate its localization and stability and affect its preference of binding partners, thus regulating autophagic activity.

2.2.1.2. PI3KC3-C2. The PI3KC3-C2 also has two-armed V-shaped tips for its membrane association, including the BARA2 domain of UVRAG instead of the BAST domain of ATG14L[67,68]. Based on the flexibility between the two arms, PI3KC3-C2 can interact with lowly curved flat endosomal membranes[67,89]. As a part of PI3KC3-C2, Beclin-1 also has a key role in various cellular trafficking processes, such as autolysosome formation, endocytic trafficking, as well as cytokinesis (Fig. 3B)[20,21,89,90]. The function of PI3KC3-C2 in autophagosome formation is controversial. Although UVRAG was reported to be involved in the early autophagy stage, reduced autophagosome formation was not observed in UVRAG-deficient cells[20,21]. PI3KC3-C2 regulates endosome trafficking by producing PI3P in intracellular membranes, which is recruited by binding to activated Rab5 *via* VPS15, thus driving early endosomal tethering and fusion[91,92]. And PI3KC3-C2 promotes the maturation of early endosomes into late endosomes by binding to Rab7 *via* VPS34 and VPS15[92]. PI3P produced by PI3KC3-C2 is necessary for the endosomal-protein-sorting role of the Retromer complex, which mediates the recycling and retrieval of cargo proteins to the trans-Golgi network or the cell surface[93]. Besides, PI3P also recruits a multi-protein complex, namely ESCRT (endosomal sorting complex required for transport), which mediates the sorting of ubiquitinated endocytic proteins towards the intraluminal vesicle of the multivesicular endosome[94]. Beclin-1 can interact with Rubicon to form PI3KC3-C2−Rubicon complex that localizes on lysosomes/late endosomes, leading to negative regulation of later events in both autophagy and endocytic trafficking[95,96]. In addition to the regulation of endocytic trafficking, the production of PI3P by PI3KC3-C2 at the furrow and midbody regions is required for successful abscission in the process of cytokinesis[90]. In addition, UVRAG association with C-Vps enhances autophagic and endocytic protein degradation independently of PI3KC3-C2 through mediating autophagosome-lysosome fusion and endosome-lysosome fusion[22].

2.2.2. *Non-autophagic functions of Beclin-1*

Recent studies demonstrate that Beclin-1 has non-autophagic functions (Fig. 3C). Although as a BH3-only protein, Beclin-1 does not function as a pro-apoptotic molecule. Interestingly, Beclin-1 can regulate autophagy and apoptosis through its caspase-mediated cleavage[47]. Caspases can lead to cleavage of Beclin-1 to generate N- and C-terminal fragments without the autophagy-inducing ability[97−99]. Only the C-terminal fragment is capable of sensitizing cells to apoptotic signals instead of the N-terminal fragment containing the BH3 domain. Beclin-1-C can translocate to the mitochondrial membrane to induce cytochrome *c* release from mitochondria[97]. Furthermore, Beclin-1-C promotes the mitochondria translocation of pro-apoptotic protein Bax to enhance apoptosis and reduce autophagy[100]. These results reveal that Beclin-1 can mediate the cross-regulation between apoptosis and autophagy, which may prove its targeting potential to regulate the two forms of cell death for therapeutic purposes.

In addition to apoptosis, Beclin-1 also plays the autophagy-independent role in other types of regulated cell death, including ferroptosis and necroptosis. AMPK (activated 5′-adenosine monophosphate-activated protein kinase)-mediated phosphorylation of Beclin-1 at Ser90, Ser93, and Ser96, which induces the Beclin-1−SLC7A11 (solute carrier family 7 member 11) interaction to directly block system Xc^- activity, thus promoting ferroptosis[101]. Furthermore, Beclin-1 has been identified as a new negative regulator of the necrosome complex. Beclin-1 interacts with the phosphorylated form of MLKL (mixed lineage kinase domain like pseudokinase) in necrosome complex, which reduces MLKL oligomerization, resulting in the inhibition of necroptosis[102]. These findings provide an insight into the autophagy-independent function of Beclin-1 in ferroptosis and necroptosis, which may accelerate its therapeutic implications in ferroptosis- or necroptosis-related diseases.

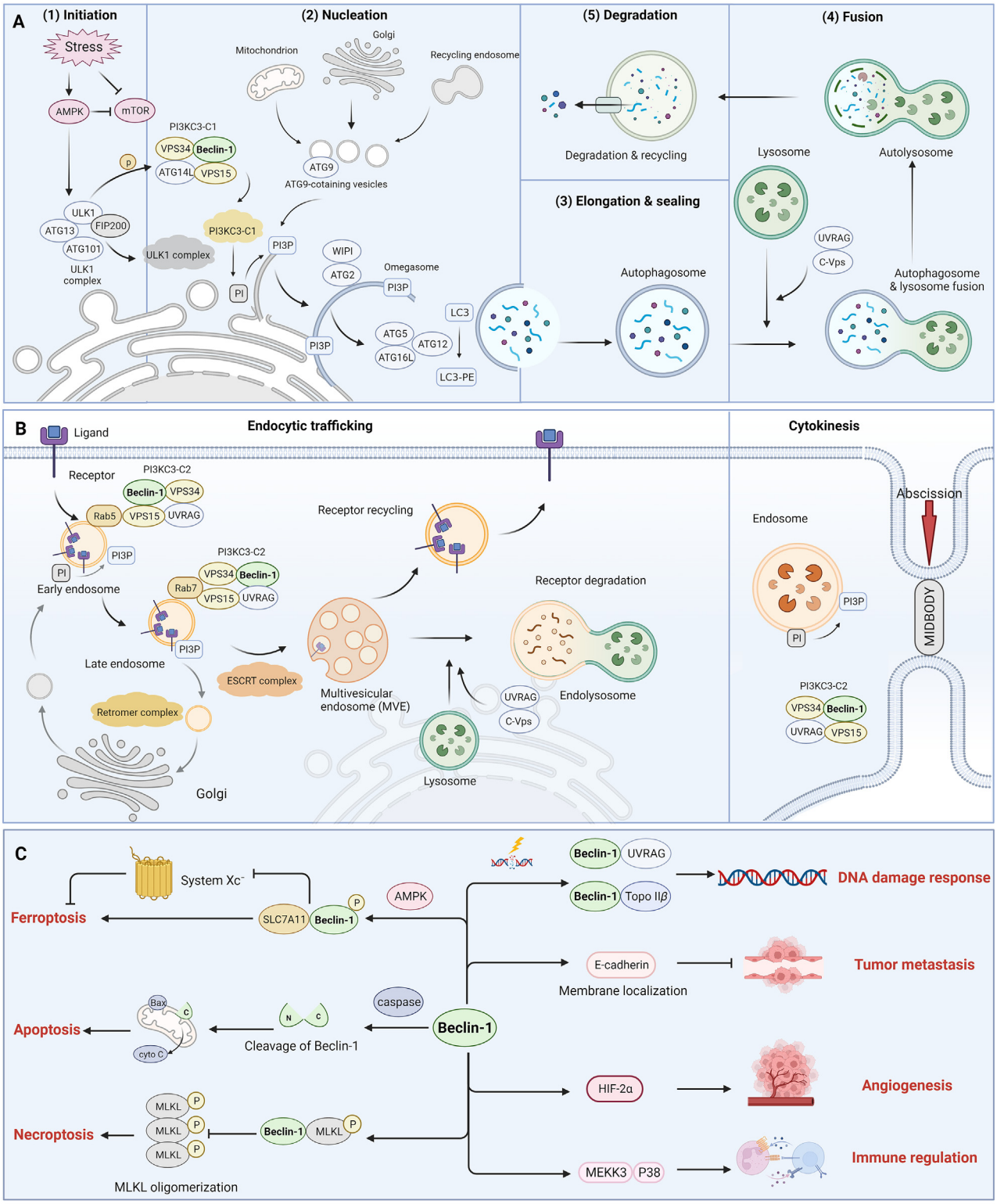


Figure 3 The functions of Beclin-1 in autophagic and non-autophagic processes. (A) The autophagy-relevant signaling pathways of Beclin-1. Upon autophagy initiation signals, PI3KC3-C1 directs PI3P production to a membrane site. The PI3P-rich membrane recruits downstream autophagy effectors, which promotes membrane expansion and cargo recruitment into the forming autophagosome. (B) As a part of PI3KC3-C2, Beclin-1 also has a pivotal role in endocytic trafficking and cytokinesis. PI3KC3-C2 regulates the fusion of early endosomes and their maturation into late endosomes. In the process of cytokinesis, PI3KC3-C2 is targeted to the midbody and produces PI3P in endosomes that cluster at the midbody during abscission. (C) Non-autophagic functions of Beclin-1. Beclin-1 has been reported to be involved in regulating other non-autophagic processes in a PI3KC3-independent manner, including apoptosis, ferroptosis, necroptosis, DNA damage response, tumor metastasis, angiogenesis, and immune regulation.

Moreover, Beclin-1 is also reported to participate in DNA damage response dependent or independent of autophagy. The Beclin-1−UVRAG interaction is capable of activating NHEJ (non-homologous end joining), which contributes to maintaining genomic stability[103]. Despite the autophagic pathway, Beclin-1 can participate in DNA damage response in an autophagy-independent manner. Nuclear Beclin-1 directly collaborates with DNA topoisomerase IIβ to repair ionizing radiation-induced DNA double-strand breaks[104].

In addition, Beclin-1 has a role in tumor metastasis, angiogenesis, and immune regulation based on its autophagy-independent functions. For instance, Beclin-1 overexpression was reported to promote the plasma membrane localization of E-cadherin in MCF7 cells through its non-autophagic function, thereby suppressing the EMT (epithelial−mesenchymal transition) and tumor metastasis[105]. In primary mouse melanoma tumor models, compare to *Becn1* wild-type mice, *Becn1* (+/−) hemizygous mice displayed an aggressive tumor growth phenotype with increased angiogenic activity *via* enhancing expression and stability of HIF-2α (hypoxia-inducible factor-2α) protein[106]. Besides, Beclin-1 overexpression was also shown to suppress angiogenesis through inhibiting the expression of vascular endothelial growth factor and matrix metalloprotease 9 *in vitro*[107]. Additionally, Beclin-1 also takes part in immune regulation[108,109]. Beclin-1 deficiency resulted in the aberrant activation of MEKK3 (mitogen-activated protein kinase kinase 3)/p38 signaling in neutrophils, which promoted B cell chemotaxis through Cxcl9−Cxcr3 axis, thus resulted in the malignant transformation of precursor B cells[109]. More details about the role of Beclin-1 in tumor metastasis, angiogenesis, and immune regulation are summarized in section 4.

3. Regulation of Beclin-1 in cancer

3.1. Transcriptional and post-transcriptional regulations of *BECN1*

The expression of the *BECN1* gene and active protein content of Beclin-1 can be controlled by various transcriptional regulations (*e.g.*, transcription factors and epigenetic alterations) and post-transcriptional regulation (*e.g.*, microRNAs, LncRNAs, and alternative splicing of Beclin-1 mRNA) (Fig. 4).

3.1.1. Transcription factors

Diverse transcription factors have been shown to regulate *BECN1* transcription *via* binding to the sites of *BECN1* promoter. The E2F transcription factors could correspond to the promoter region for *BECN1* to participate in the regulation of its expression. As one of NF-κB (nuclear factor kappa B) family members, P65 binds and activates the *BECN1* promoter, thus up-regulating *BECN1* expression, highlighting an autophagy-related mechanism whereby p65 could promote cell survival[110,111]. Recently, X-linked inhibitor of apoptosis and cellular inhibitor of apoptosis 1 are two key inhibitors of apoptosis proteins that could activate NF-κB and induce autophagy by upregulating the transcription of *BECN1*, which might be associated with the chemotherapy resistance in several human cancers[112]. Conversely, TRIM59 (tripartite motif containing 59 protein) acts as a negative modulator of the NF-κB pathway, attenuating the transcription of *BECN1* gene, thus regulating autophagy in non-small cell lung cancer (NSCLC)[113]. GABP (GA binding protein) could activate the transcriptional activity of *BECN1* gene and autophagosome initiation in case of nutrient starvation[114]. FOXM1 (Forkhead Box M1) directly binds to the promotor regions and enhances the promoter activity of *BECN1* gene and regulates their expression, thus enhancing autophagy and promoting survival of triple-negative breast cancer cells[115]. KLF5 (Krüppel-like factor 5) is transcription factor that could bind and inhibit *BECN1* promoter collaboratively with HDAC3 (histone-deacetylase 3), resulting in the suppression of *BECN1* transcription[116]. The downregulation of KLF5 increases *BECN1* expression and induces cell autophagy in prostate cancer cells, leading to desensitization to docetaxel[116].

3.1.2. Epigenetic alteration

Epigenetic alterations that involve modification of chromatin structure has been shown to be associated with the transcriptional regulation of *BECN1* gene. As the human *BECN1* gene contains a 1.5 kb CpG island from its 5′ end to the intron 2, which can be aberrantly methylated in breast cancer, resulting in the decreased expression of *BECN1*[117]. EHMT2/G9a (euchromatic histone-lysine *N*-methyltransferase 2) can increase amounts of dimethylation of lysine 9 on histone H3 that binds to the *BECN1* promoter and repressed the transcription of *BECN1* gene through an epigenetic mechanism[118]. Besides, treatment with BIX-01294 (an EHMT2 inhibitor) could reverse the suppression of Beclin-1 by the action of EHMT2, resulting in autophagy induction in breast cancer MCF7 cells[118].

3.1.3. MicroRNAs

MicroRNAs (miR) can function as post-transcriptional regulator that influence protein expression of Beclin-1 *via* suppressing protein synthesis or degrading mRNA. MiR-30a/376b/221 can directly down-regulate the translational levels of Beclin-1 in several types of human cancers, which attenuate rapamycin (an autophagy inducer that up-regulates *BECN1*)-induced autophagy[119−122]. MiR-216a and miR-409-3p can interact with the 3′-untranslated region of *BECN1* mRNA, which inhibit Beclin-1 synthesis and reduce autophagic activity, involving in the radio- or chemo-resistance in the treatment of human cancers[123,124]. MiR-26a negatively regulates autophagy by reducing *BECN1* mRNA in human retinoblastoma cell[125]. MiR-17-5p directly targets *BECN1* mRNA and suppresses irradiation-induced autophagy mediated by Beclin-1 in the glioma[126]. MiR-124-3p could negatively regulate the expression of Beclin-1in breast cancer cells[127]. Recent studies revealed that the expression of miR-93/21/1301/216 b showed the inverse correlation with the expression of Beclin-1 and inhibits autophagic activity in glioblastoma, bladder cancer, ovarian cancer, and melanoma, respectively[128−131].

3.1.4. LncRNAs

Long non-coding RNAs (lncRNAs) participate in regulating a variety of biological processes *via* affecting gene expression[132,133], and the regulatory functions of several lncRNAs in *BECN1* gene expression have been revealed. LncRNA *H19* can up-regulate the *BECN1 via* inhibiting SAHH (*S*-adenosylhomocysteine hydrolase) to decrease DNMT3B (DNA methyltransferase 3 B)-mediated methylation of *BECN1* promoter, thus increasing autophagy activity and promoting tamoxifen resistance in breast cancer[134]. Overexpression of lncRNA *PANDAR* can promote *BECN1* expression at the transcription and translation levels in lung cancer, thus inhibiting the development of lung cancer[135]. Overexpression of lncRNA *FIRRE* can induce the

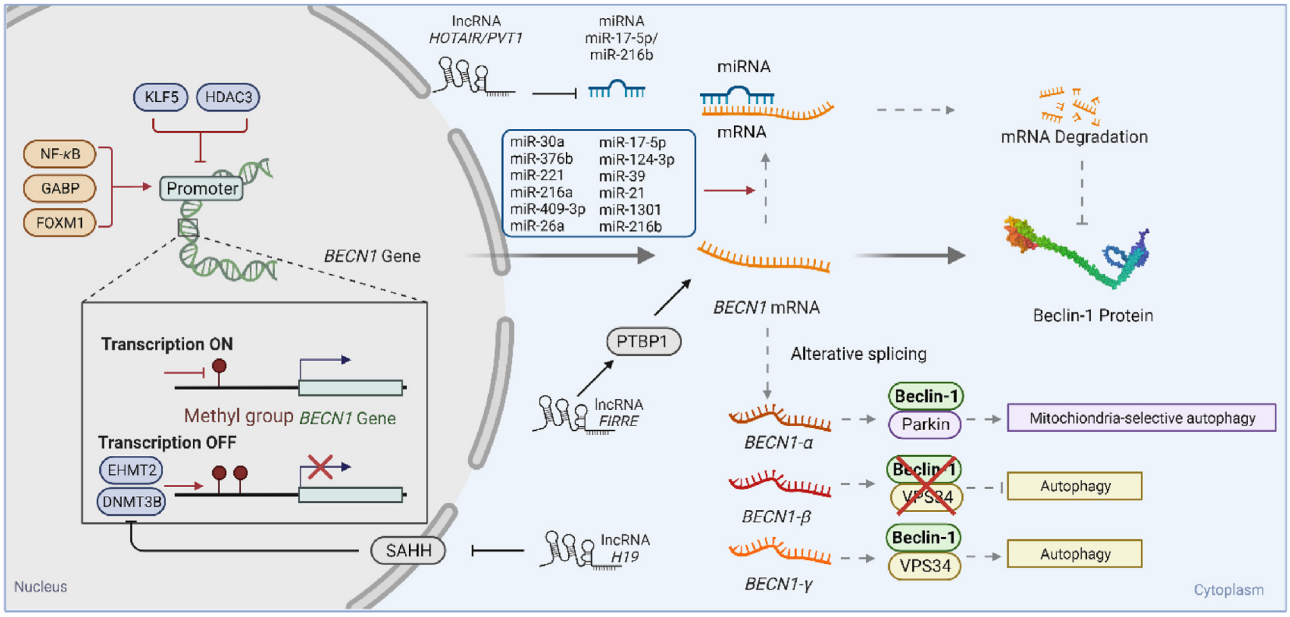


Figure 4 Transcriptional and post-transcriptional regulation of *BECN1*. Diverse transcription factors can regulate *BECN1* transcription *via* interacting with *BECN1* promoter. NF-κB, GABP, and FOXM1 promote *BECN1* transcriptional activation, while KLF5 and HDAC3 suppress the transcription of *BECN1*. Additionally, EHMT2 and DNMT3B participate in the transcription of *BECN1* gene through epigenetic mechanisms. Various microRNAs can influence the post-transcription of *BECN1* through promoting the degradation of its mRNA, and lncRNAs can regulate *BECN1* expression *via* stabilizing it mRNA, affecting its epigenetic modification, or sponging to the regulatory miRNAs. Furthermore, alternative splicing of *BECN1* mRNA leads to different splice variants of Beclin-1 products with different structures and functions.

translocation of PTBP1 (polypyrimidine tract-binding protein) from the nucleus to the cytoplasm, which consequently stabilizes *BECN1* mRNA and facilitate autophagy in colorectal cancer[136]. Moreover, lncRNA is known as a competing endogenous RNA (ceRNA) by sponging to miRNAs to modulate the expression of these miRNA targets. For example, lncRNA *HOTAIR* as a ceRNA for miR-17-5p can reduce miR-17-5p to promote the *BECN1* expression, thus enhancing autophagy in renal cancer cells[137]. Similarly, lncRNA *PVT1* can function as a ceRNA for miR-216 b to regulate *BECN1* expression in lung cancer cells[138].

3.1.5. *Alternative splicing*

The alternative splicing mechanism of mRNA enables cells to produce diverse variants from a specific gene[139,140]. Recent reports reveal that *BECN1* mRNA undergoes alternative splicing due to the splicing consensus sequences in the *BECN1* gene. A *BECN1* transcript variant with a deletion of exon 11 was found and its translational protein showed an attenuated activity in starvation-induced autophagy, indicating that alternative splicing of *BECN1* mRNA may serve as a negative autophagic regulator[141]. Through the alternative splicing mechanism, a novel splice variant of *BECN1*, namely *BECN1s/BECN1-α*, whose protein product can bind to Parkin and have a function in mitochondria-selective autophagy[141,142]. Recently, two novel mRNA splicing variants of *BECN1*, called *BECN1-β* and *BECN1-γ*, were identified in human ovarian cancer cells[142]. The product of *BECN1-β* isoform lacks the BH3 domain as well as part of the CCD and ECD compared to functional Beclin-1, compromising its ability to interact with VPS34, leading to the inhibition of autophagy[142]. The product of *BECN1-γ* lacks the BH3 domain as well as part of the CCD and BARA domain, still maintaining its capacity to interact with VPS34 and thus showing a minor effect on autophagy[142]. The discovery of diverse *BECN1* splice variants indicates the complex regulatory mechanisms of *BECN1*. It is hypothesized that the composition and respective expression level of these isoforms in cancer cells might be regulated to modulate autophagy and mitophagy under particular environmental stimulus, including starvation or hypoxia. More investigations are required to elucidate the regulation mechanism of alternative splicing on *BECN1* and to understand the role of *BECN1* splice variants in autophagy.

3.2. *Post-translational modifications of Beclin-1*

Various post-translational modifications (*e.g.*, phosphorylation, ubiquitination, ISGylation, SUMOylation, acetylation, and cleavage) can modulate Beclin-1 function to affect autophagy and other Beclin-1-involved cellular processes (Fig. 5).

3.2.1. *Phosphorylation*

Phosphorylation is a crucial modification of Beclin-1 involved in the induction or inhibition of the autophagy process[48]. The upstream signaling initiates a phosphorylation cascade *via* loss of mTORC1 (mammalian target of rapamycin complex 1) activity[143]. mTORC1 as a master nutrient sensor can sense amino-acid and nutrient starvation and thus activates the ULK1 complex and initiates autophagy process[144]. Activated ULK1 phosphorylates the N-terminal of Beclin-1 at Ser15 or Ser30, which can subsequently activate PI3KC3-C1 and promote the induction of autophagy[77,145]. Moreover, PGK1 (phosphoglycerate kinase 1) also mediates the activating phosphorylation of Beclin-1 at Ser30, which specifically promotes cell proliferation and brain tumorigenesis under hypoxic conditions[146]. In addition, under cellular energy-depletion conditions, AMPK can regulate the activity of

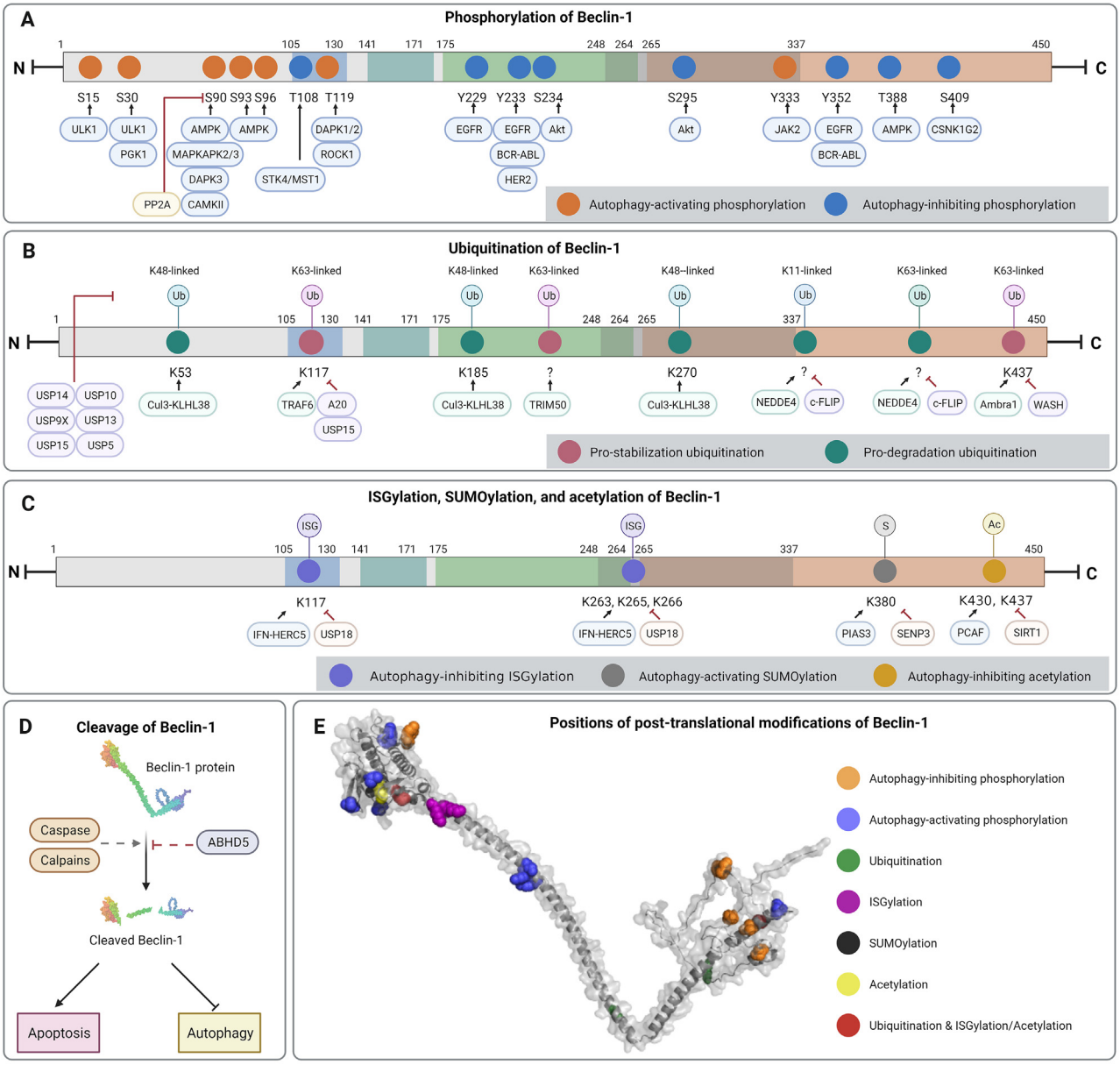


Figure 5 Post-translational modifications of Beclin-1. (A) Phosphorylation of Beclin-1. Phosphorylation-modified residues that lead to the inhibition of autophagy are indicated in orange, and those with autophagy-activating effects are indicated in blue. (B) Ubiquitination of Beclin-1. Ubiquitination-modified residues that promote the degradation of Beclin-1 are indicated in green, and those with pro-stabilization effects are indicated in red. (C) ISGylation, SUMOylation, and acetylation of Beclin-1. ISGylation-modified residues and acetylation-modified residues that both inhibit autophagy are indicated in purple and yellow, respectively. The SUMOylation-modified residue that induces autophagy is indicated in grey. (D) Cleavage of Beclin-1. Beclin-1 is a direct substrate of caspase and calpains in apoptosis, and the cleavage of Beclin-1 can be inhibited by ABHD5. Cleaved Beclin-1 is sufficient to suppress autophagy and promote apoptosis. (E) Positions of post-translational modifications of Beclin-1. Beclin-1 is visualized and oriented in a structure model based on the crystal structure of Beclin-1 predicted by AlphaFold (https://alphafold.ebi.ac.uk/entry/Q53F78).

the autophagic components, including ULK1 and Beclin-1[147,148]. AMPK also phosphorylates the N-terminal of Beclin-1 at Ser90 and Ser93, resulting in the autophagy induction[77,149]. Interestingly, AMPK has a dual role in autophagy process *via* phosphorylating Beclin-1 at different sites. AMPK can phosphorylate the BARA domain at Thr388, which inhibits the PI3KC3 formation *via* suppressing the Beclin-1−VPS34 interaction[148]. Besides, AMPK-mediated phosphorylation of Beclin-1 at Ser90, Ser93, and Ser96 can promote ferroptosis *via* inducing the Beclin-1−SLC7A11 interaction[101]. Furthermore, Ser90 of Beclin-1 can be phosphorylated by other kinases, such as MAPKAPK2/3 (mitogen-activated protein kinase-activated protein kinases 2/3)[150,254], DAPK3 (death-associated protein kinase 3)[151], and CAMKII (Ca^{2+}/calmodulin-dependent protein kinase II)[152], leading to autophagy initiation. PP2A (protein phosphatase 2A) also directly dephosphorylates Ser90 of Beclin-1 and okadaic acid

(a PP2A inhibitor) could induce Ser90 phosphorylation under non-starved conditions[151]. As members of the DAPK family, DAPK1 and DAPK2 can phosphorylate Beclin-1 at Thr119 (a key residue within the BH3 motif), which can impede the Beclin-1−Bcl-X_L/Bcl-2 interaction, thus promoting the activation of the autophagic machinery[153−155]. Upon metabolic stress, the serine/threonine ROCK1 (Rho kinase 1) can bind and phosphorylate Beclin-1 at Thr119, which also reduces Beclin1−Bcl-2 association and enhances Beclin-1-induced autophagy[156]. Recently, a study reported that the STK4/MST1 (serine/threonine kinase 4) could phosphorylate Beclin-1 within its BH3 motif at Thr108, which could increase the binding affinity of Beclin-1 and Bcl-X_L/Bcl-2[157]. The serine/threonine kinase Akt phosphorylates Beclin-1 at Ser234 and Ser295, leading to autophagy inhibition, which demonstrates a cross-talk between oncogenic kinases and autophagy proteins[158]. The CSNK1G2 (casein kinase I gamma 2) phosphorylates Beclin-1 at Ser409, which is helpful for the Beclin-1−p300 interaction to promote Beclin-1 acetylation, thus governing Beclin-1 function in autophagosome maturation and tumor growth[159]. Tyrosine kinases (TKs), including receptor TKs (RTKs) and non-receptor TKs (NRTKs), can phosphorylate substrates at tyrosine residues, which have been reported to participate in Beclin-1 phosphorylation to modulate autophagy process. EGFR (epidermal growth factor receptor), an oncogenic RTK, mediates multisite tyrosine phosphorylation (Tyr229, Tyr233, and Tyr352) of Beclin-1, thus suppressing autophagy activity and affects endocytic trafficking, which may contribute to tumor progression and chemoresistance to TK inhibitors[160]. HER2 can induce tyrosine phosphorylation at Tyr233 similar to active EGFR that also inhibits Beclin-1 function to promote HER2-mediated tumorigenesis[46]. The BCR−ABL is an active constitutive TK that directly binds and mediates Beclin-1 tyrosine phosphorylation at Tyr233 and Tyr352, which suppresses autophagy induction by inhibiting the formation of PI3KC3 and enhancing interaction between the negative interactome Rubicon and Beclin-1[161]. Furthermore, the BCR−ABL suppresses Beclin-1-mediated autophagy and thereby bypasses the negative effect of autophagy on tumor cell survival and proliferation[161]. As another crucial NRTK protein, JAK2 (Janus kinase 2) interacts with Beclin-1 induced by Interleukin-6 (IL-6) and phosphorylates it at Tyr333, leading to enhanced Beclin-1−VPS34 interaction and increased autophagy activity[162]. This study demonstrates that the IL-6−JAK2 axis activates Beclin-1-mediated autophagy by inducing Beclin-1 Tyr333 phosphorylation and promotes chemotherapy resistance in colorectal cancer[162].

3.2.2. *Ubiquitination and deubiquitination*

Ubiquitination not only functions as a signal for the degradation of targeting protein, but also participates in the regulation of the localization, composition, or activity of multiprotein complexes[163−165]. In the process of ubiquitination of Beclin-1, ubiquitin molecules will be covalently attached to its lysine residues through a cascade catalyzed by E1, E2, and E3 enzymes. NEDD4 (neural-precursor-cell-expressed developmentally downregulated 4) is an E3 ubiquitin ligase of Beclin-1 that could modify Lys11- and Lys63-linkage ubiquitination, resulting in its degradation[166]. Without any known catalytic activities, c-FLIP (cellular FLICE-like inhibitory protein) functions as a scaffold protein that binds to Beclin-1 and masks key residues of Beclin-1 recognized by NEDD4, thus preventing NEDD4-mediated ubiquitination and degradation of Beclin-1[167]. Additionally, the Cul3 (Cullin3) ubiquitin ligase is involved in Lys48 ubiquitination of Beclin-1 at K53, K185, and K270, and KLHL38 (Kelch-like protein 38) plays a role in the recognition and interaction of CUL3 and Beclin-1 in breast cancer cells[168]. Cul3-KLHL38 facilitates the ubiquitination and degradation of Beclin-1 and inhibits autophagic activity, leading to tumor progression of breast cancer[168]. TRIM50 could ubiquitinate Beclin-1 in Lys63-dependent manners, thus promoting the PI3KC3 formation and enhancing the autophagy activity [113,169]. TRAF6 (tumor necrosis factor receptor-associated factor 6) also can induce Lys63-linked ubiquitination that has a pivotal role in TLR4 (Toll-like receptor 4)-induced autophagy[170,171]. The deubiquitinating enzyme A20 could inhibit the autophagy induction in response to TLR signaling by directly deubiquitinating Beclin-1 and limiting the TRAF6-mediated Lys63-linked ubiquitination[170,171]. CAMKII-mediated Ser90 phosphorylation of Beclin-1 can increase the TRAF6-mediated Lys63-linked ubiquitination of Beclin-1, leading to activation of autophagy in ionomycin/EB1089 treated neuroblastoma cells[152]. Moreover, USP15 (ubiquitin-specific protease-15) regulates the TRAF6−Beclin-1 signaling axis by inducing deubiquitination of Beclin-1, thereby attenuating autophagy induction and negatively regulating lung cancer progression (migration and invasion) induced by TLR4 stimulation[172]. Ambra1 is an E3 ligase that also contributes to lys63-linked ubiquitination to induce Beclin-1-dependent autophagy under starvation conditions[173]. WASH (Wiskott-Aldrich syndrome protein and SCAR homologue) could competitively bind Beclin-1 to impede its ubiquitination, resulting in inactivated VPS34 activity and autophagy suppression[173]. Solute carrier family 9 subfamily A member 3 regulator 1 could bind to Beclin-1 and subsequently blocks ubiquitin-dependent Beclin-1 degradation, thereby stimulating autophagy and suppressing breast cancer cell proliferation[174]. Recently, lysine-specific demethylase 2 A has been shown to mediate the ubiquitination as well as degradation of Beclin-1, thus inhibiting autophagy and promoting CircRNF144B−miR-342-3p axis-mediated ovarian cancer progression[175].

As a deubiquitinating enzyme, USP14 negatively controls Lys63-linked ubiquitination of Beclin-1, thus regulating the process of autophagy[176,177]. Moreover, USP14 knockdown in human breast carcinoma MDA-MB-231 cells and human hepatic adenocarcinoma SK-HEP-1 cells resulted in increased cell migration and invasion, indicating that USP14 is negatively implicated in the cancer progression by inhibiting Beclin-1 ubiquitination[177]. Moreover, USP9X, USP10, and USP13 have been confirmed as deubiquitinases that can mediate the deubiquitination of Beclin-1, thus modulating autophagy activity[178,179]. Recently, Kras-mediated USP5 activation is reported to deubiquitinate K48-linked polyubiquitination of Beclin-1 and stabilize Beclin-1, resulting in autophagy and p53 protein instability, thereby promoting Kras-driven lung tumor growth[180].

3.2.3. *ISGylation and deISGylation*

Similar to ubiquitylation, ISGylation is a reversible process, in which conjugates a ubiquitin-like modifier, namely ISG15 (interferon-stimulated gene 15 protein), to a substrate protein[181]. The expression of ISG15 induced by Type I IFN (interferons) can mediate ISGylation of Beclin-1 at Lys117, Lys263, Lys265, as well as Lys266[182]. Furthermore, the ISGylation of Beclin-1 competes for its Lys63-linked polyubiquitination, leading to the inhibition of PI3KC3 activity and autophagy flux[182]. Moreover, HERC5 (HECT and RLD domain containing 5 protein) is an E3 enzyme that could interact with Beclin-1 and catalyze ISGylation of Beclin-1[183]. USP18 could remove conjugated ISG15 from Beclin-1, thus positively regulating autophagy[182]. These findings

may provide a novel mechanistic insight into the link between immunity and autophagy implemented by ISGylation and deISGylation of Beclin-1. However, further research is needed to elaborate on the roles of ISGylation and deISGylation of Beclin-1 in oncology.

3.2.4. *SUMOylation and deSUMOylation*

As another post-translational modification process, SUMOylation dynamically attaches a SUMO (small ubiquitin-like modifier) to a lysine residue of a target molecule, which can affect various molecular pathways[184]. PIAS3 (protein inhibitor of activated signal transducer and activator of transcription 3) has been identified as a SUMO E3 ligase for Beclin-1 that amplifies the SUMO3 conjugates predominantly at Lys380 of Beclin-1 under cellular starvation conditions, which induces autophagy *via* facilitating PI3KC3 complex formation and promoting PI3KC3 activity[185]. Conversely, deSUMOylation of Beclin-1 could be mediated by SUMO-specific peptidase SENP3 (SUMO-specific protease 3), which suppresses autophagy *via* impairing PI3KC3 complex formation and inhibiting PIK3C3 activity in human liver, breast, colorectal, and cervical carcinoma cell lines[185]. The reversible SUMOylation of Beclin-1 by PIAS3 and SENP3 provides a fine-tuning mechanism for tumor cells to regulate autophagy to cope with different situations.

3.2.5. *Acetylation and deacetylation*

As a reversible protein modification process, lysine acetylation can reversibly alter the structure as well as the function of proteins, therefore, is involved in almost all cellular processes[186]. Lysine acetylation has been reported as a novel regulatory mechanism affecting the structure and function of Beclin-1, thus inhibiting autophagosome maturation and autophagy induction. Beclin-1 can be acetylated by PCAF (p300/CBP-associated factor) at Lys430 and Lys437, which favors its interaction with Rubicon that alters the composition of PI3KC3 complex and hampers autophagosome maturation[159]. In contrast, SIRT1 (sirtuin 1) can deacetylate Beclin-1, leading to attenuated Beclin-1−Rubicon interaction[159]. Moreover, decreased acetylation of Beclin-1 by the mutation of K430 and K437 sites in MCF7 xenografts results in enhanced autophagosome maturation, decreased cellular proliferation and tumor growth, demonstrating that an acetylation-dependent regulatory mechanism of Beclin-1 promotes tumor growth[159].

3.2.6. *Cleavage*

Beclin-1 is demonstrated as a target of the cleavage mediated by caspase or calpain. Its protein levels can be controlled by proteolysis-dependent mechanism to regulate its autophagic functions. The cleavage of Beclin-1 mediated by caspase has been reported to link the autophagic and apoptotic pathways[187,188]. Caspase can cleave the polypeptide chain of Beclin-1 into fragments, thereby abrogating its autophagic function and enhancing the apoptotic pathway[97–99,189,190]. ABHD5 (abhydrolase domain containing 5 protein), a cellular lipolytic activator, can compete with Caspase-3 for binding to the cleavage sites of Beclin-1, thus preventing its cleavage by Caspase-3[191]. Moreover, calpains has been shown to be responsible for Beclin-1 cleavage at a specific site between BH3 and CCD domain, leading to the inhibition of autophagy[192,193].

Overall, various transcriptional and post-transcriptional regulations of *BECN1* gene that can affect its expression level and as well as splice variants, as well as post-translational modification of Beclin-1 proteins that can affect its stability, interactions and functions, have been well revealed as essential regulatory mechanisms to fine-tune the process of autophagy. However, it is still waiting for further researches to determine how different regulations affect each other and how regulations diverge under diverse conditions. Besides, the post-translational modification of Beclin-1 also provides the cell with a novel strategy to regulate other processes, such as apoptosis and ferroptosis. More investigations on the regulation of Beclin-1 are required to deepen our understanding of its regulatory roles in non-autophagic processes.

4. The role of Beclin-1 in cancer

Hitherto, alterations of *BECN1* gene and its products Beclin-1, including mutation status of *BECN1*, monoallelic deletion of *BECN1,* as well as altered expression profiles of Beclin-1 protein expression, have been reported in diverse cancers (Table 1). Given the complexity of autophagy, Beclin-1 shows complicated roles in different subtypes, stages, and genetic contexts of cancers. On one hand, Beclin-1 acts as a haplo-insufficient tumor suppressor, as its monoallelic deletion or decreased expression is associated with tumorigenesis and tumor progression. However, on the other hand, Beclin-1 may also have a role in the progression of tumors by supporting tumor cell survival under cellular stress (Fig. 6A)[194,195]. Besides, diverse non-autophagic functions of Beclin-1 make its roles in cancer more complex. In addition, autophagy contributes to the drug resistance of tumor cells, the homeostasis of CSCs, as well as the regulation of TME (Fig. 6B and C).

4.1. *The oncosuppressive role of Beclin-1*

Beclin-1 has been implicated as a tumor suppressor, activating autophagy and preventing tumorigenesis. Monoallelic deletion of *BECN1* or loss of Beclin-1 expression has been widely reported to favor the occurrence and progress of numerous types of malignant tumors. Heterozygous disruption of the *BECN1* gene leads to a high incidence of spontaneous malignant tumors (*e.g.*, hepatocellular and lung carcinomas, as well as B cell/lymphoblast cell lymphomas) in mice models[25,234]. Additionally, *Becn1* heterozygosity allows the survival of the immortalized baby mouse kidney epithelial cells possessing an apoptotic defect during starvation, and Beclin-1 haploinsufficiency promotes epithelial tumorigenesis[235]. These results indicate that Beclin-1 serves as an oncosuppressive regulator in tumorigenesis.

4.1.1. *Autophagy-related role*

Beclin-1-mediated autophagy has been shown to function as an important tumor-suppressive mechanism. Firstly, Beclin-1-mediated autophagy can limit the progress of malignant tumors by inducing tumor cell death. Compared to wild-type mice, $Becn1^{F121A/F121A}$ knock-in mice with the disrupted Beclin-1−Bcl-2 interaction exhibited enhanced basal autophagic flux, resulting in a lower incidence of age-associated spontaneous malignancies[236]. Beclin-1 overexpression results in reduced cell proliferation, enhanced apoptosis, and blocked cell cycle in colorectal cancer HT29, HCT-15 and HCT-116 cell lines[218,237], glioblastoma U87 cells[238], cervical cancer HeLa cells[239], as well as gastric cancer MKN-45 cells[240], whereas down-regulating Beclin-1 causes increased proliferation of colorectal cancer HCT116 and SW620 cell lines[241] as well as human lung cancer A549 cells[242].

Table 1 The alterations of the *BECN1* gene and Beclin-1 expression in cancers clinically.

Cancer types	Alterations of *BECN1*/Beclin-1	Clinical correlation	Ref.
Ovarian cancer	Monoallelic deletion of *BECN1* (~75%)	Shallow deletion of *BECN1* with low mRNA expression shows higher sensitivity to platinum-based therapies and is associated with better overall and disease-free survival.	196–200
Ovarian cancer	Loss or down-regulation of Beclin-1 protein expression	Loss or decreased expression of Beclin-1 is correlated with ascending histological grade, advanced stage, poor progression-free, and shorter overall survival.	26,201,202
Breast cancer	The mutation (IVS1−4 T > A) of *BECN1* was detected in 1 of 94 breast cancers (1.0%).	/	203
Breast cancer	Monoallelic deletion of *BECN1* gene (~50%)	/	204,205
Breast cancer	Down-regulated Beclin-1 protein and mRNA expression	Decreased Beclin-1 expression is correlated with a poor 5-year overall survival rate and poor distant metastasis-free survival of ER-positive, HER2-negative breast cancer.	27,28
Breast cancer	Beclin-1 is differentially expressed according to the molecular subtype of breast cancer.	High-level Beclin-1 expression was related to TNBC-type tumors, which have high nuclear pleomorphism and a high mitotic index.	206
Prostate cancer	Monoallelic deletion of *BECN1* (~40%)	/	23
Prostate cancer	Down-regulated Beclin-1 protein expression	Beclin-1 expression is negatively correlated with the Gleason score, and decreased expression of Beclin-1 may collaboratively represent an aggressive malignant phenotypic feature of prostate carcinoma cells.	29
Gastric cancer	The mutations (24 C > A, 1165 C > T, IVS1−4 T > A, and IVS5+7 C > T) of *BECN1* were detected in 5 of 180 gastric cancers (2.8%).	/	203
Gastric cancer	Down-regulated Beclin-1 protein and mRNA expression	Beclin-1 expression is positively linked to the favorable prognosis of patients, and decreased Beclin-1 expression is associated with poor differentiation, advanced stage, and shorter overall survival.	30,207,208
Gastric cancer	Up-regulated Beclin-1 protein and mRNA expression	Increased Beclin-1 expression is related to better disease-free survival and longer overall survival, with a smaller tumor size, mixed histologic type, better histological grade, lower recurrence rate, less lymphatic, vascular, and neural invasion.	209–212
Gastric cancer	/	Positive Beclin-1 expression is correlated with lymph node metastasis, vessel invasion, hepatic metastasis, and poor survival.	213
Colorectal cancer	The mutations (1049 C > G and IVS5+7 C > T) of *BECN1* was detected in 3 of 50 colorectal cancers (2.8%).	/	203
Colorectal cancer	Beclin-1 is differentially expressed: underexpression (15.5%), the normal-like pattern (40.6%), limited overexpression (23.2%), and extensive overexpression (21.3%).	Extensive Beclin-1 overexpression is significantly linked with nodal involvement, high histological grade, and vascular invasion.	42,214
Colorectal cancer	Up-regulated Beclin-1 protein and mRNA expression	Increased Beclin-1 expression is positively correlated with histological grade and clinical stage related, with better disease-free survival and longer overall survival, and it is negatively related to liver and distant metastasis.	209,215–218
Oral squamous cell carcinoma	Down-regulated Beclin-1 protein expression	Decreased Beclin-1 expression is correlated with poor differentiation, lymph node metastasis, advanced clinical tumor-node-metastasis stage, and a poor prognosis.	31
Oral squamous cell carcinoma	/	Increased Beclin-1 expression is correlated with the degree of tumor infiltration.	219

Table 1 (*continued*)

Cancer types	Alterations of *BECN1*/Beclin-1	Clinical correlation	Ref.
Lung cancer	The mutation (IVS1−4 T > A) of *BECN1* was detected in 1 of 124 lung cancers (0.8%).	/	203
NSCLC	Down-regulated Beclin-1 protein expression	Decreased Beclin-1 expression is associated with higher tumor recurrence rate, more advanced stages, poor overall survival, and poor progression-free survival, with more lymph node metastasis and more poorly differentiated tumors.	220,221
Hepatocellular carcinoma	Down-regulated Beclin-1 protein and mRNA expression	Decreased Beclin-1 expression is related to tumor recurrence, poor differentiation, and poor prognosis.	32,222−224
Cervical cancer	Down-regulated Beclin-1 protein expression	Decreased Beclin-1 expression is associated with pelvic lymph node metastasis and histological grade.	33
Cholangiocarcinoma	Down-regulated Beclin-1 protein expression	Decreased Beclin-1 expression is correlated with lymph node metastasis and poor 3-year progression-free survival.	34
Chondrosarcoma	Down-regulated Beclin-1 protein and mRNA expression	Decreased Beclin-1 expression is correlated with increasing tumor grade and poor overall survival.	35
Brain cancer	Down-regulated Beclin-1 protein and mRNA expression	Decreased Beclin-1 expression is correlated with higher-grade brain cancer.	36,37
Meningiomas	/	High-level expression of Beclin-1 is correlated to better prognosis, lower pathological grade, and longer survival.	225
Melanoma	Down-regulated Beclin-1 protein and mRNA expression	Decreased Beclin-1 expression is associated with tumor progression and distant metastasis.	38,226,227
Lymphoma	Down-regulated Beclin-1 protein and mRNA expression	Decreased Beclin-1 expression is associated with poor overall survival and progression-free survival	39,228
Hypopharyngeal squamous cell carcinoma	Down-regulated Beclin-1 protein and mRNA expression	Decreased Beclin-1 expression is correlated with poor differentiation and more lymph node metastasis.	40
Esophageal squamous cell carcinoma	Loss of Beclin-1 protein expression in 33% of esophageal squamous cell carcinomas.	Beclin-1 expression is negatively correlated with the depth of invasion, lymph node metastasis, and clinical stage.	229,230
Pancreatic ductal adenocarcinoma	Up-regulated Beclin-1 protein expression	Increased Beclin-1 expression is correlated with the absence of lymphatic invasion and a low rate of distant metastasis	231
Nasopharyngeal carcinoma	/	Increased Beclin-1 expression is correlated with poorer overall survival, progression-free survival, and distant metastasis-free survival.	232
Papillary thyroid carcinoma	Up-regulated Beclin-1 protein expression	Increased Beclin-1 expression is significantly correlated with tumorigenesis and lymph node metastasis.	233

Enforced Beclin-1 expression in breast cancer MCF-7 cells can inhibit cell proliferation *in vitro* and suppress tumor growth *in vivo* in MCF-7 xenograft nude mice models[17]. Beclin-1 overexpression induces autophagy and suppresses the proliferation of esophageal cancer Eca109 cell line, and inhibits the growth of Eca109 xenograft tumor in nude mice[243]. Furthermore, Beclin-1-mediated autophagy conduces to reducing DNA damage and maintaining chromosomal stability, thus preventing tumorigenesis. *BECN1* heterozygosity impairs the autophagy activity of immortalized mouse mammary epithelial cells, which is related to DNA damage accumulation (the gamma phosphorylated form of the histone H2AX foci) in immortalized mouse mammary epithelial cells, ultimately promoting mammary tumorigenesis[244]. Importantly, Beclin-1 cooperated with UVRAG could regulate the DNA damage pathways and centrosome stability of colorectal cancer HT29 cells in an autophagy-independent manner, and Beclin-1 knockdown sensitizes cells to DNA damage and apoptosis[103].

4.1.2. *Autophagy-independent role*

Increasing evidence provided several autophagy-independent molecular mechanisms contributing to the tumor-restraining potential of Beclin-1. For instance, Beclin-1 affects the p53 level through modulating the deubiquitination activity of USP10 and USP13, and loss of Beclin-1 may promote tumorigenesis by decreasing the levels of p53[179]. Besides, Beclin-1 negatively regulates the proteasomal degradation of the tumor promoter Mcl-1 through competitively displacing USP9X[178,245]. Besides, co-regulation of decreased Beclin-1 and subsequently increased Mcl-1 is associated with melanoma progression[178]. Beclin-1 also involves in the regulation of the specific trafficking function, endosome maturation, and immunosuppressive function to inhibit tumorigenesis, which is the oncosuppressive role of Beclin-1 beyond autophagy. *BECN1* heterozygosity can lead to augmented mammary stem and progenitor cell activity and aberrant up-regulation of the NF-κB activator, namely tumor necrosis factor receptor superfamily member 11a,

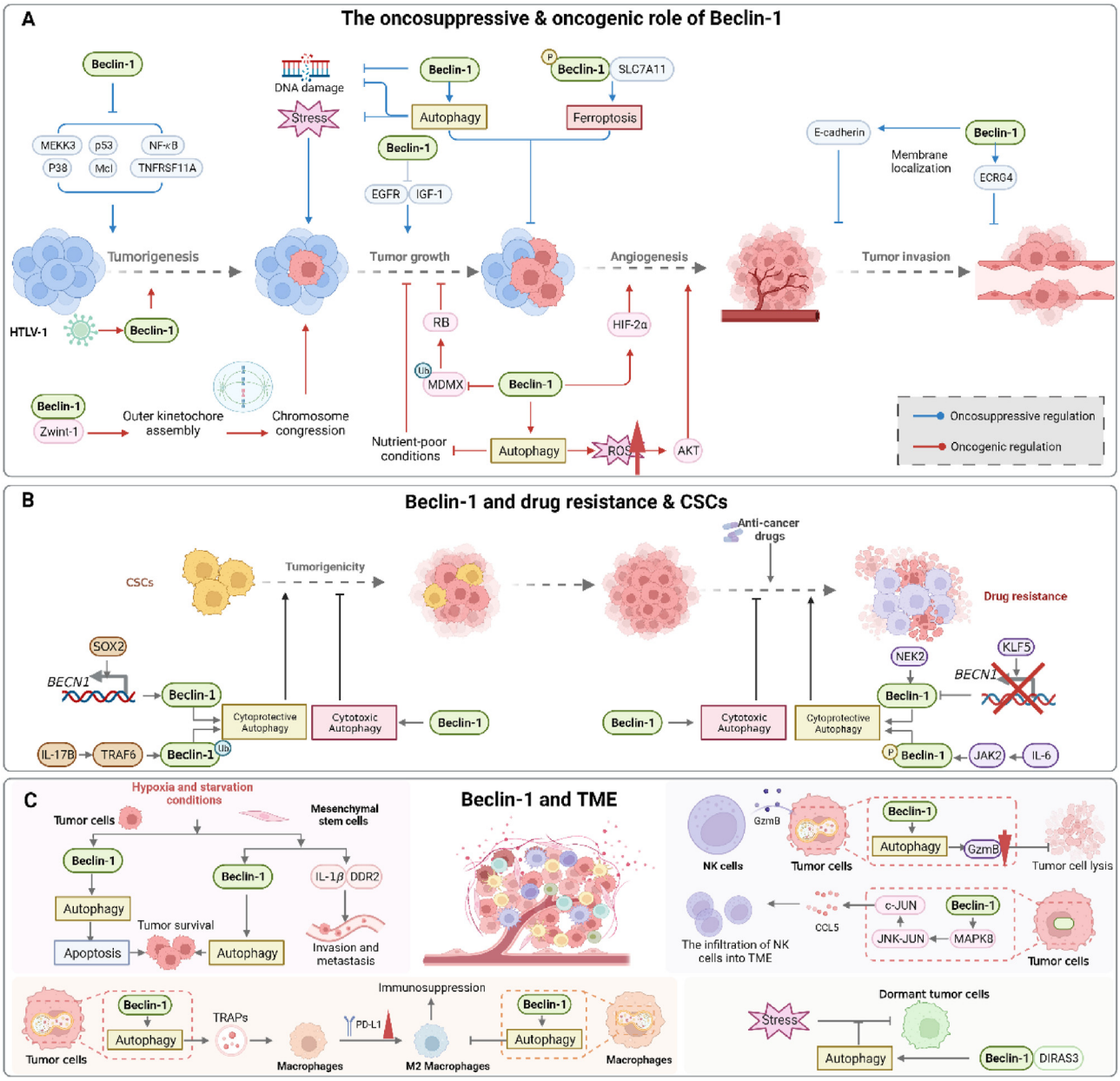


Figure 6 Illustration of the role of Beclin-1 in cancer. (A) Beclin-1 functions as an oncosuppressive regulator or an oncogenic regulator through its autophagic or non-autophagic roles. (B) Beclin-1 contributes to the development or elimination of drug resistance to cancer therapy, and Beclin-1-induced autophagy has discrepant roles in CSC stemness maintenance. (C) Beclin-1 serves as a multifaceted regulator of TME, as it shows dual effects of promoting and inhibiting cancer.

thus promoting spontaneous mammary tumorigenesis following parity in FVB/N mice[246].

Recently, it is reported that Beclin-1 could increase E-cadherin plasma membrane localization in MCF7 cells to promote cell−cell adhesion and down-regulation of beta-catenin/Wnt target genes and mesenchymal markers, thereby suppressing the EMT as well as inhibiting tumor growth and progression[105]. Moreover, Beclin-1 contributes to controlling a specific stage of early endosome maturation, which involves in negatively regulating the intensity and duration of both EGFR and IGF-1 (insulin-like growth factor-1) signaling[160,247,248]. Loss of Beclin-1 has a potential role in enhancing these signaling pathways in breast carcinoma cells, leading to the activation of oncogenic drivers, including Akt and ERK (extracellular signal-regulated kinase), thus promoting migration or invasion of breast cancer[160,248]. Besides, Beclin-1 can regulate the endocytic trafficking and degradation of the growth factor and nutrient receptors (EGFR and transferrin receptor) to suppress tumor proliferation[249]. Moreover, overexpression of Beclin-1 could markedly decrease invasion and induce apoptosis in A549 cells through up-regulation of the tumor suppressor ECRG4 (esophageal cancer-related gene 4)[250].

Additionally, Beclin-1 has been reported to serve as a neutrophil-specific immune checkpoint, providing an autophagy-independent mechanism for neutrophil-driven carcinogenesis[108,109]. Myeloid-deficient *Becn1* mice were shown to develop neutrophilia with a high incidence of spontaneous precursor B cell

lymphoma[109]. Beclin-1 deficiency resulted in the aberrant activation of MEKK3/p38 signaling in neutrophils, which promoted B cell chemotaxis through the Cxcl9−Cxcr3 axis[109]. The interaction of Beclin-1-deficient neutrophils and B cells could further continuously activate oncogenic signaling, including CD40L/ERK and IL-21/STAT3/IRF1, and contributed to the increased expression of PD-L1 (programmed death ligand 1), thus promoting the malignant transformation of precursor B cells[109]. This study offers evidence to support that Beclin-1 involves in B cell carcinogenesis beyond its role in autophagy and might be a promising target for cancer immunotherapy.

Furthermore, Beclin-1 is currently reported to play a novel role in regulating system Xc^- activity by binding to SLC7A11, which is pivotal for inducing ferroptosis, and Beclin-1 overexpression obviously strengthens the anti-tumor activity of erastin (a ferroptosis inducer) by increasing ferroptosis *in vitro* and *in vivo*[101,251].

The progress of research on the potential anticarcinogenic mechanisms of Beclin-1 is described to illustrate its suppressive role in tumorigenesis. Although the specific mechanism by which Beclin-1 hinders tumorigenesis is still unclear, there is mounting evidence that Beclin-1 modulates autophagy-dependent and -independent pathways, which constantly changes our understanding of its oncosuppressive potential. Therefore, further research is needed to delineate the oncosuppressive mechanisms of Beclin-1 in autophagy and beyond.

4.2. *The oncogenic role of Beclin-1*

Several reports have shown the controversial result that Beclin-1 can function as an oncogenic molecule associated with tumorigenesis and tumor progress. Increased expression of Beclin-1 can be correlated with oncogenesis, vessel invasion, or lymph node metastasis of a variety of cancers, including breast, gastric, nasopharyngeal, and papillary thyroid carcinomas, and can be linked with these cancer patients' poor overall survival as well as progression-free survival and distant metastasis-free survival[206,213,232,233]. In addition, Beclin-1 knockdown in human triple-negative breast cancer cells could inhibit tumor growth, migration, and invasion by inducing G0/G1 cell cycle arrest and incompletely repressing the EMT of tumor cells[252]. Similarly, Beclin-1 knockdown impairs EMT of colon cancer SW620 and LOVO cells[253]. In Ewing sarcoma SK-ES-1 cells, knocking down Beclin-1 suppresses cell proliferation, invasion, and migration by inhibiting matrix metalloprotease 9[254]. Moreover, Beclin-1 overexpression enhances the migratory ability of NSCLC (non-small cell lung cancer) cells by affecting the ubiquitination of Vimentin (a mesenchymal marker), and Beclin-1 knockdown significantly inhibits cell migration[255]. These studies reveal that Beclin-1 has an oncogenic role in tumor growth, migration, and invasion.

4.2.1. *Autophagy-related role*

The oncogenic roles of Beclin-1 are primarily associated with its autophagy-inducing function, which can support tumor cells to survive under nutrient-poor or hypoxic conditions. For instance, Beclin-1-mediated autophagy could promote the formation of VM (vasculogenic mimicry) induced by hypoxia in glioma by generating reactive oxygen and activating Akt. Silencing Beclin-1 by siRNA not only significantly inhibited hypoxia-induced VM formation in U87MG cells but also hampered migration and invasion of U87MG cells, suggesting its positive role in the progress of glioma[256]. Additionally, Beclin-1 contributes to the aberrant proliferation of HTLV-1 (human T cell leukemia virus type 1)-transformed T cells by inducing the cytoprotective autophagy and maintaining the persistent activity of NF-κB and STAT3, which are two key pro-survival factors associated with the pathogenesis of HTLV-1-mediated oncogenesis[257].

4.2.2. *Autophagy-independent role*

Beclin-1 also exerts its oncogenic role in an autophagy-independent manner. Beclin-1 has been reported to participate in chromosome congression by promoting proper outer kinetochore assembly through its interaction with Zwint-1 (the subunit of the structural kinetochore)[258]. The depletion of Beclin-1 remarkably decreases the outer kinetochore proteins in HeLa cells, such as CENP-E/F and ZW10[258]. As a consequence, the chromosome congression of these Beclin-1-depleted HeLa cells would be negatively affected[258]. Recently, it is reported that Beclin-1 is elevated in the nucleus in malignant human colorectal tumor specimens, which is negatively correlated with Retinoblastoma expression[259]. Silencing of Beclin-1 facilitates MDM2 and MDMX (murine double minute 2 and X) complex formation to promote MDMX polyubiquitination and degradation, thus up-regulating and stabilizing Retinoblastoma, leading to growth suppression of HCT-116 cells independent of p53[259]. Besides, Beclin-1 knockdown significantly inhibits tumor growth in HCT-116 xenograft models by activating Retinoblastoma[259].

The oncogenic role of Beclin-1 in the tumorigenesis and progress of several cancer types has been investigated, while it is urgent to establish an intact mechanism framework. Despite of the controversial role of Beclin-1 in cancer pathology, it is undeniable that Beclin-1 is a promising molecular target for the treatment of cancer. These opposite findings reveal that the Janus functions of Beclin-1 may attribute to the differential expression of itself and its effector molecules or its major subcellular localization in distinct tumor cells.

4.3. *Beclin-1 and drug resistance to cancer therapy*

Beclin-1 has also been shown to involve in the resistant mechanism to chemotherapy and targeted cancer therapy in diverse tumors. The differential expression of Beclin-1 is shown between drug-sensitive and -resistant cell lines. For instance, the expression levels of Beclin-1 and other ATG proteins were down-regulated in oxaliplatin-resistant colon cancer SNU-C5 cells than in oxaliplatin-sensitive SNU-C5 cells[260]. On the contrary, compared with normal human hypopharyngeal squamous cell carcinoma FaDu cell line, autophagy, and Beclin-1 expression were enhanced in cisplatin-resistant FaDu cells[261]. These competing findings demonstrate that Beclin-1 also plays a dual role in resistance to cancer therapy.

Recent studies revealed that regulating Beclin-1 might contribute to overcoming resistance to cancer therapy. Compared to the Enzalutamide-sensitive prostate cancer cells, the expression of Beclin-1 was decreased in Enzalutamide-resistant cells, and ectopically expressed Beclin-1 in the resistant cells led to the significantly increased Enzalutamide-sensitivity[262]. Compared with etoposide/cisplatin-sensitive groups, Beclin-1 expression was up-regulated in etoposide/cisplatin-resistant small cell lung cancer H446 and Letp cells and patient tissues and Beclin-1 inhibition reversed the *in vitro* chemoresistance of etoposide/cisplatin-resistant H446 and Letp cells[263]. Moreover, Beclin-1 significantly increased and autophagosome formation was increased in the TRAIL (TNF-related apoptosis-inducing ligand)-resistant

colon cancer DLD1 cells, and Beclin-1 knockdown restores the response to TRAIL in resistant DLD1 cells[264]. Beclin-1 knockdown increased imatinib sensitivity in gastrointestinal stromal tumors GIST-T1 and GIST-882 cells, and miR-30a could sensitize these cells to imatinib *via* down-regulating Beclin-1 and inhibiting autophagy[265]. Besides, Beclin-1 expression was significantly increased in the oxaliplatin-resistant colorectal cancer cells compared with the parental cells, and miR-409-3p could enhance the cell chemosensitivity to oxaliplatin by suppressing Beclin-1-mediated autophagy[124].

Additionally, Beclin-1 also participates in the development of resistance caused by the dysfunction of its upstream regulatory molecules. For example, IL-6 can induce the JAK2−Beclin-1 interaction, consequently, leading to the phosphorylation of Beclin-1 at Tyr333 to induce autophagy, a mechanism by which the IL-6/JAK2/Beclin-1 signaling pathway regulates chemotherapy drug resistance in colorectal cancer[162]. Downregulation of KLF5 results in elevated Beclin-1 expression and enhanced autophagy activity in prostate cancer cells, which results in decreased drug sensitivity to docetaxel *in vitro* and *in vivo*[116]. A serine/threonine kinase NEK2 (never in mitosis-related kinase 2), can stabilize Beclin-1 by promoting the deubiquitination mediated by USP7, consequently conferring drug resistance in multiple myeloma[266]. These inspiring findings suggest that targeting Beclin-1 could be a promising strategy to reverse the resistance to chemotherapy as well as targeted therapy for cancer patients.

4.4. *Beclin-1 and CSCs*

Similar to stem cells, CSCs allow the self-renewal of cells and the generation of differentiated cells, thus promoting cell survival and malignancy[267,268]. Several studies indicate that Beclin-1 contributes to the self-renewal of CSCs, contributing to the tumorigenicity of CSCs. Beclin-1 expression is increased at both mRNA and protein levels with the elevated autophagic flux in breast CSCs/progenitor cells and depletion of Beclin-1 in breast CSCs/progenitor cells reduces the incidence of *in vivo* xenograft formation, which suggests that Beclin-1 is essential for the tumorigenicity of CSCs[43,269]. The interaction between Beclin-1 and TRAF6 can be induced and enhanced by the IL-17B/IL-17RB (interleukin-17 B and its corresponding receptor) signaling cascade in gastric CSCs, leading to ubiquitination of Beclin-1 and autophagy induction, which further enhance the stemness and tumorigenesis ability of gastric CSCs *in vitro* and promote tumor growth and invasion *in vivo*[270]. SOX2 (sex-determining region Y-box2), a core regulator of embryonic, activates autophagy by transcriptional activation of Beclin-1 and drives CSCs properties in colorectal cancer[271]. Most importantly, Beclin-1 knockdown could partially diminish SOX2-driven malignant phenotypes in colorectal cancer SW480 cells, demonstrating the involvement of Beclin-1 in SOX2-induced CSCs properties in colorectal cancer[271]. These studies reflect that Beclin-1 seems to conduce to the maintenance and tumorigenicity of CSCs.

On the other hand, given the suppressive role of pro-death autophagy in cancer progression, several studies have reported conflicting results that autophagy enhancement reduces the self-renewal of CSCs, induces differentiation of CSCs, and promotes therapy sensitivity[272,273]. Currently, a study reported the disruptive effects of Beclin-1 on CSCs maintenance. Silencing *BECN1* by short hairpin RNA (shRNA) results in the inhibition of autophagy on glioblastoma CSCs, which enhances the expression of stemness markers and promote proliferation and clonogenicity of glioblastoma CSCs[274]. These findings suggest that Beclin-1 knockdown reinforces the stemness of glioblastoma CSCs and awakens them from the dormant state[274].

In conclusion, the published literature reveals that Beclin-1-induced autophagy has discrepant roles in CSC stemness maintenance, suggesting that there is a more complex relationship between autophagy and CSCs. Additionally, it remains to demonstrate whether the non-autophagy functions of Beclin-1 are involved in the regulation of CSCs. Further investigations are required to explore the molecular mechanism of Beclin-1 in CSCs maintenance, which may provide potential molecular targets to eliminate CSCs for better cancer therapy.

4.5. *Beclin-1 and TME*

Tumor microenvironment (TME) has a pivotal role in cancer biology, which is composed of tumor cells and various stromal cell populations (*e.g.*, fibroblasts, endothelial cells, neutrophils, macrophages, and adipocytes), as well as extracellular-matrix components, soluble mediators, and cytokines produced by these cells[275]. The TME can inhibit host antitumor immunity, promote the transformation, growth, and invasion of tumors, and facilitate therapeutic resistance and dormant metastases[276]. Since autophagy has crucial functions in cross-talk between tumor cells and TME[277], Beclin-1 as a key autophagy regulator also contributes to regulating TME.

Due to the poor blood supply in solid tumors, decreased available oxygen and insufficient nutrient supply widely exist in TME. Hypoxia-induced autophagy can result in apoptosis reduction in a Beclin-1-dependent way, which enhances the tolerance of tumor cells to nutrient deprivation. Silencing *BECN1* by the small interfering RNA (siRNA) significantly abrogated such apoptosis reduction and tolerance to nutritional deprivation in hepatocellular carcinoma cells, suggesting that Beclin-1-mediated autophagy facilitates tumor survival under hypoxia and starvation conditions in TME[278]. Moreover, the upregulation of autophagy in mesenchymal stem cells can support tumor cell survival under the nutrient-deprived condition[279]. Additionally, the expression of Beclin-1 in mesenchymal stromal cells contributes to cancer invasion and metastasis[280]. SiRNA-mediated downregulation of Beclin-1 can decrease the expression of IL-1β and collagen receptor DDR2 (discoidin domain receptor 2) in mesenchymal stromal cells, which can foster tumor invasiveness by shaping the TME[280].

Natural killer (NK) cells function as the fundamental effector cells in antitumor innate immunity[281]. Hypoxia-induced autophagy in breast cancer MCF-7 cells can degrade NK-derived GzmB (granzyme B) to reduce susceptibility to NK-mediated tumor cell lysis *in vitro*, and knocking down *BECN1* to inhibit autophagy in MCF-7 cells can restores intracellular GzmB level[282]. Furthermore, inhibition of autophagy by silencing *BECN1* can induce tumor regression in two aggressive syngeneic murine models: B16-F10 melanoma tumors and 4T1 breast carcinoma tumors[282]. This study elucidates the mechanism by which Beclin-1-mediated autophagy protects tumor cells from NK-mediated killing. A recent report reveals another pro-tumorigenic mechanism of Beclin-1 *via* suppressing the infiltration of NK cells into TME[283]. Knocking down *BECN1* in mouse melanoma B16-F10 cells lead to the activation of the MAPK8/JNK-JUN/c-Jun signaling pathway, which promotes the overexpression and release of CCL5 cytokine in the TME to induce a massive infiltration of NK cells into TME, thus suppressing

melanoma growth by breaking the immunosuppressive TME barrier[283]. This study provides a novel therapeutic strategy based on inhibiting Beclin-1 in tumor cells to improve the infiltration of NK cells to the TME and execute their cytotoxic function against cancer cells within the TME.

Macrophages that infiltrate TME can be driven by tumor-derived secretions to acquire a polarized M2 phenotype, which is regarded as a pro-tumor macrophage phenotype[284]. Tumor cell-released autophagosomes (TRAPs) isolated from multiple murine tumor cell lines and pleural effusions or ascites of cancer patients could mediate immunosuppression in TME by increasing PD-L1 expression to polarize macrophages towards a tumor-promoting (M2-like) phenotype[285]. Silencing *BECN1* by shRNA in the murine melanoma cell line B16F10 could cause the reduction of TRAPs secretion with attenuated ability to induce PD-L1 expression in macrophages, supporting that Beclin-1 is crucial for the formation of TRAPs and participates in the polarization of tumor-associated macrophages[285]. Interestingly, Beclin-1-mediated autophagy in macrophages can suppress M2 macrophage polarization. Silencing *BECN1* by siRNA in mouse RAW 264.7 macrophages could efficiently restrain autophagy activity and increase the polarization of RAW 264.7 to M2 macrophages[286]. The results of the two studies reveal the reverse roles of Beclin-1-mediated autophagy in the polarization of macrophages that might depend on different microenvironments and different types of cells.

In addition, tumor dormancy is defined as an adaptive mechanism to stress conditions within the TME. The Beclin-1−DIRAS3 interaction is responsible for the survival of dormant ovarian cancer cells *via* the induction of autophagy to face nutrient-deprived stress[63]. This study steers a rational design strategy of DIRAS3-derived peptide that aims to inhibit Beclin-1 for eliminating ovarian tumors[287].

Overall, Beclin-1 serves as a multifaceted regulator of TME, as it shows dual effects of promoting and inhibiting cancer. Additionally, the roles of Beclin-1 differ in the diverse cell types in the TME, making it hard to predict the exact outcome of targeting Beclin-1 for cancer therapy. Therefore, more detailed investigations are needed to elucidate the specific role of Beclin-1 in tumor cells, immune cells, and other stromal cells. And given the complexity of the TME, therapeutic targeting Beclin-1 requires much caution and refinement.

5. Targeted Beclin-1-regulating strategies for cancer therapy

Although mutations of the *BECN1* gene are notably rare in cancers, monoallelic losses of the *BECN1* gene have been observed in many human cancers according to haploinsufficiency network analyses. Given its widespread deregulation and crucial role in tumorigenesis and tumor progression, Beclin-1 has become a potential therapeutic target to develop novel anti-tumor strategies. Moreover, non-homozygous copy number losses of the *BECN1* gene contribute to maintaining the autophagy pathway in cancer cells, which allows cells to tolerate stress, thus conferring resistance to antitumor therapy. However, as a scaffolding protein, targeting Beclin-1 had long been thought impossible. Despite being traditionally referred to as “undruggable”, considerable progress has recently been made in targeted pharmacological regulation of Beclin-1 for cancer therapy. In this section, the current targeted Beclin-1-regulating strategies, including targeted peptides, small molecules, and other strategies are summarized (Fig. 7).

5.1. Peptides

Currently, Tat-Beclin-1 has been reported to be applied for the inhibition of tumorigenesis and the treatment of cancers[46,101,288]. Tat-Beclin-1 was shown to disrupt HER2−Beclin-1 interaction in four HER-positive breast cancer cell lines BT-474 cells, BT-474-VH2 cells, SK-BR3 cells, and MDA-MB-361 by inducing a strong autophagic flux[46].

Based on the structure of the CCD of Beclin-1, several hydrocarbon-stapled peptides were designed to specifically interact with the CCD of Beclin-1 with a high affinity[289]. Among these designed peptides, Tat-SP4 (Fig. 7A) significantly inhibits the self-association of Beclin-1 and enhances its interaction with Atg14L/UVRAG to induce autophagy and enhance endolysosomal degradation. More importantly, Tat-SP4 shows the anti-tumor effect against the proliferation of cancer cells *via* activating the function of Beclin-1, thus promoting autophagy and endolysosomal degradation of EGFR[289]. As the suppression mechanism is orthogonal to that employed by EGFR-TKIs (tyrosine kinase inhibitors), Tat-SP4 synergized with erlotinib (an EGFR-TKI) could significantly inhibit the proliferation of A549 and H1975 cells by enhancing their sensitivity to erlotinib[290]. Recently, through staple scanning and sequence permutation, these peptides were optimized to make the hydrocarbon staple closer to the interface of CCD, thus improving the affinity to the target protein Beclin-1[291]. Compared to the prototype peptide Tat-SP4, the optimized peptide i7-01s-31 (Fig. 7B) showed approximately 10-fold higher binding affinity to target protein Beclin-1 and approximately 5-fold more potent inhibitory efficacy in EGFR-overexpressed and HER2-positive breast cancer SKBR3 cell line[291]. However, i7-01s-31 inhibits the proliferation of SKBR3 cells *via* inducing necrotic cell death instead of apoptosis, and the molecule mechanism of such necrotic cell death is required to further investigate whether it is induced by enhanced autophagy induction or increased EGFR and HER2 degradation[291]. Given the pro-survival role of Beclin-1 in dormant ovarian cancer cells, a Beclin-1-inhibiting peptide, named Tat-D3S2, was designed based on the switch II region of DIRAS3 (residues 93−107), which could be taken up by ovarian cancer cells by linking to Tat peptide (Fig. 7C)[287]. The results show that Tat-D3S2 inhibits amino acid deprivation-induced autophagy by the selective disruption of the Beclin-1−DIRAS3 interaction in ovarian cancer cells[287]. However, further detailed research is required to evaluate its therapeutic potential to eliminate dormant ovarian tumors.

Beclin-1-activating peptides (Tat-Beclin-1, Tat-SP4, and i7-01s-31) have been shown to have the activities to regulate the protein-protein interactions of Beclin-1 to enhance autophagy and promote the endolysosomal degradation of EGFR or HER2, thus inhibiting the proliferation of EGFR- or HER2-driven cancer cells. Furthermore, Beclin-1-targeting peptide Tat-D3S2 inhibits autophagy through the selective disruption of protein-protein interaction (Beclin-1−DIRAS3) critical for autophagosome initiation, thus eliminating dormant ovarian tumors that express DIRAS3. In conclusion, the development of Beclin-1-targeting peptides has demonstrated the feasibility of regulating a specific protein−protein interaction of Beclin-1 critical to its autophagic and non-autophagic functions. However, due to the low proteolytic and conformational stability of peptides, their clinical efficacy may be limited. More studies are needed to characterize the thermodynamics and kinetics of these Beclin-1-targeting peptides, which may further inform the design of more potent and stable peptides for clinical application.

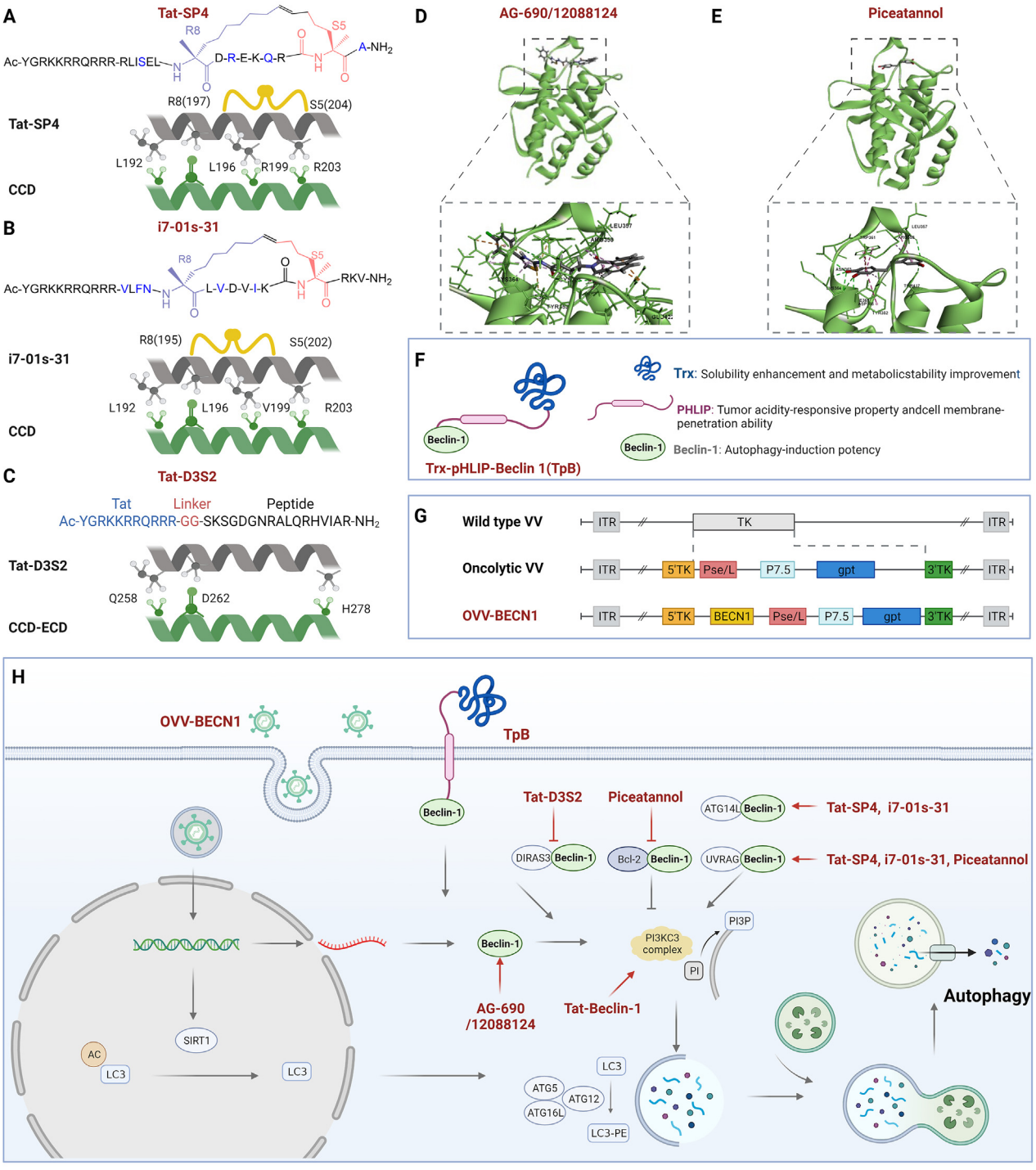
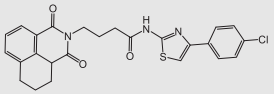
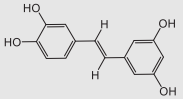


Figure 7 Illustration of targeted Beclin-1-regulating strategies for cancer therapy. (A, B) Sequence and chemical structure of (A) Tat-SP4 and (B) i7-01s-31 and their predicted binding modes to Beclin-1 CCD. Modified residues are colored in blue, and residues colored in black remain unchanged. R8 and S5 represent two residues that were chemically modified to form the hydrocarbon linkage. (C) The sequence of Tat-D3S2 and its predicted binding sites to Beclin-1 CCD and ECD. (D, E) The binding mode of Beclin-1 together with (D) AG-690/12088124 and (E) piceatannol. The two small-molecule Belcin-1 activators superimposed in the active site of the ECD of Beclin-1 (PDB ID:4DDP). (F) TpB is composed of three different components, which endow it with some unique characteristics. TpB could specifically accumulate in weakly acidic tumors (pH 6.5) and effectively deliver Beclin-1 to cancer cells by forming an α-helix across the plasma membrane. (G) Schematic diagram of the recombinant OVV-BECN1 structure. *BECN1* full-length gene was inserted into the TK-flanking regions of the shuttle plasmid pCB, and homologous recombination was occurring between pCB-Beclin1 and wild-type VV in HEK 293 cells. The promoter was Pse/L and the screen gene was the gpt gene. (H) Schematic demonstration of the mechanism of these targeted Beclin-1-regulating strategies.

Table 2 Targeted Beclin-1-regulating strategies for cancer therapy.

Name	Sequence/Structure information	Target domain	Function	Biological activity	Cancer type	Ref.
Tat-Beclin-1	YGRKKRRORRR-GG-TNVENATEEIWHDGEFGT	ECD	Inducting autophagy	Tat-Beclin-1 (16 mg/kg) showed a similar *in vivo* inhibitory effect as lapatinib (100 mg/kg) on mice HER2-positive BT-474-VH2 xenografts.	HER2-positive human breast cancer	46
Tat-SP4	YGRKKRRQRRR-RLISEL (R8)DREKQR(S5)A	CCD	Enhancing autophagy and the endolysosomal degradation of EGFR	A549 (IC_{50} = 50.64 μmol/L) H1975 (IC_{50} = 43.54 μmol/L)	EGFR-overexpressed NSCLS	289
i7-01s-31	YGRKKRRQRRR-VLFN (R8)LVDVIK(S5)RKV	CCD	Enhancing autophagy and endolysosomal trafficking	SKBR3 (IC_{50} = 7.12 μmol/L)	EGFR- or HER2-driven breast cancer	291
Tat-D3S2	YGRKKRRORRR-GG-SKSGDGNRALQRHVIAR	CCD-ECD	Inhibiting autophagy	Tat-D3S2 inhibited the cell viability of A2780 and SKOV3 during amino acid deprivation by inhibiting autophagy.	Ovarian Cancer	287
AG-690/12088124		ECD	Inducting autophagy	MDA-MB-468 (IC_{50} = 9.01 ± 2.31 μmol/L) MDA-MB-231 (IC_{50} = 8.25 ± 1.53 μmol/L) MCF-7 (IC_{50} = 14.25 ± 1.82 μmol/L) BT-549 (IC_{50} = 12.21 ± 1.03 μmol/L)	Breast cancer	292
Piceatannol		ECD	Inducting autophagy	C7901 (IC_{50} = 2.3 μmol/L) BGC823 (IC_{50} = 3.1 μmol/L) MKN28 (IC_{50} = 7.3 μmol/L)	Gastric cancer	293
TpB	Trx tag-AAEQNPIYWARYADWLFTTP LLLLDLALLVDADEGT-CGTNVFNATFHIWHSGQFGT	/	Inducting autophagy	MCF-7 (IC_{50} = 12.79 μmol/L, pH = 6.5) SKOV3 (IC_{50} = 16.73 μmol/L, pH = 6.5)	Breast and ovarian cancer	295
OVV-BECN1	/	/	Inducting autophagy	OVV-BECN1(20 MOI) induces autophagic cell death in K562 and U266 hematologic malignant cells	Leukemia and myeloma	299

5.2. *Small molecules*

Through docking-based virtual screening, AG-690/12088124 (Fig. 7D) was identified as a small-molecule candidate that could activate Beclin-1 through binding to the ECD[292]. Additionally, AG-690/12088124 showed an inhibitory effect on the proliferation of human breast cancer cell lines, including MDA-MB-468, MDA-MB-231, MCF-7, and BT-549 cells, with IC_{50} values of approximately 10 μmol/L[292]. Moreover, AG-690/12088124 induced autophagic cell death and apoptosis in MDA-MB-231 cells[292]. The results suggest that the candidate AG-690/12088124 could be a lead compound for further developing and optimizing small-molecule activators of Beclin-1 to regulate autophagy in tumor cells for therapeutic purposes[292]. Recently, piceatannol (Fig. 7E) was confirmed as a promising Beclin-1-targeting agonist, which could directly bind to ECD and reduce Beclin-1 phosphorylation at Ser295[293]. Piceatannol markedly repressed the proliferation of human gastric cancer SGC7901, BGC823, and MKN28 cell lines. Notably, piceatannol not only impaired the Beclin-1−Bcl-2 interaction but also enhanced the Beclin-1−UVRAG binding, thus initiating autophagy in gastric cancer cells. Moreover, its combination with an mTOR inhibitor, everolimus, showed a more potent inhibitory effect on SGC7901 and MGC803 cells as well as an anti-tumor effect on the SGC7901 xenograft models, confirming that piceatannol can elicit a synergistic effect with everolimus for gastric cancer therapy[293].

With much lower molecular weights, small-molecule drugs are more suitable for targeting intracellular proteins. However, specific pharmacological targeting of Beclin-1 with small-molecule compounds is difficult, since human Beclin-1 protein possesses an intrinsically disordered structure (residues 1−150) and the crystal structure of full-length Beclin-1 is not elucidated. Fortunately, the structure of Beclin-1 C-terminal half-encompassing ECD has been reported at 1.6 Å resolution[66], which provides important clues for the discovery of Beclin-1-targeting small-molecule compounds (AG-690/12088124 and piceatannol). Furthermore, the stunning success of the artificial intelligence-powered AlphaFold that is able to predict the structure of every protein, raised drug discovery hopes[294].

5.3. *Others*

In addition, several studies reveal that the techniques of biomedical engineering can be used to directly increase the intracellular content of Beclin-1 protein in targeted tumor cells. It is reported that the extracellular pH of normal tissues is 7.2−7.4, whereas that of tumor tissues can be more acidic (6.2−6.8)[295]. Trx-pHLIP-Beclin-1 (TpB) has been reported to be a functional Beclin-1 that was constructed to provide a novel delivery manner for cancer therapy[295]. TpB protein consists of a thioredoxin (Trx) tag, a pH low insertion peptide (pHLIP), and an evolutionarily conserved motif of Beclin-1, which aims to translocate Beclin-1 protein to cancer cells through forming a stable transmembrane α-helix under a weakly acidic environment (pH 6.5) (Fig. 7F)[295]. TpB could markedly suppress the growth and proliferation of ovarian cancer SKOV3 and breast cancer MCF-7 cell lines by inducing autophagic cell death. Moreover, TpB protein showed a preferential and selective accumulation at tumor sites other than various healthy tissues and retard the tumor growth of SKOV3 xenograft-bearing tumor mouse models *via* autophagy activation *in vivo*[295]. Inserting a therapeutic gene into the OVVs (oncolytic vaccinia viruses) genome to induce cell death has emerged as a potential therapeutic strategy for patients with hematological malignancies[296−298]. Therefore, a new OVV harboring *BECN1* gene (OVV-BECN1) was constructed to induce autophagic cell death in human leukemia K562 and HL-60 as well as multiple myeloma U266 cell lines (Fig. 7G)[299]. The results indicated that OVV-BECN1 induced autophagic cell death by up-regulating SIRT1, which further led to LC3 deacetylation that promotes its cytoplasmic distribution from the nucleus, thereby initiating the formation of the autophagosome. Furthermore, OVV-BECN1 displays a potent anti-leukemia activity on K562 xenograft mouse models[299]. These findings suggest that the virotherapy-based modality provides a promising Beclin-1-regulating strategy for the treatment of blood cancers.

Biologically synthesized tumor acidity-responsive Beclin-1 protein (TpB) and OVV armed with Beclin-1 (OVV-BECN1) are two unconventional approaches for the design of novel Beclin-1-targeting agents, which are also promising for cancer therapy. More importantly, the introduction of pHLIP provided an exceptional low-pH targeting property to deliver theranostic Beclin-1 to acidic TME[295], which will inspire further research to develop more effective tumor-targeted delivery approaches of various therapeutic agents. OVV-BECN1 resulted in an enhanced anti-tumor therapeutic effect by combining OVV-based virotherapy and Beclin-1-based gene therapy[299], suggesting that combinational treatment with Beclin-1-targeting agents may improve the therapeutic efficacy of the OVV in preclinical or clinical trials.

In conclusion, Beclin-1-targeting regulation has emerged as a potential therapeutic strategy for certain types of tumors (Fig. 7H, Table 2). As indicated, regulating *BECN1* expression or Beclin-1 activity has been proven to inhibit tumor growth in several preclinical tumor models. These inspiring findings provide novel insights into the potential of Beclin-1-targeting treatment for future cancer therapeutic intervention. In addition, other newly-emerging techniques, such as proteolysis targeting chimera (PROTAC) and noncoding RNAs, may further inspire researchers to better develop Beclin-1-targeting cancer therapy[132,300−302]. Moreover, considering the various other functions of Beclin-1 in immunity, neuroprotection, and lifespan extension, the above-summarized Beclin-1-targeting agents might be pleiotropic for preventing or treating other human diseases[303].

6. Conclusions and perspectives

As a major catabolic process, autophagy is evolutionarily conserved in eukaryotes, which is closely related to human diseases, especially cancers. Beclin-1 is a well-established autophagy inducer that is reported as a tumor suppressor in diverse malignant tumors. However, with in-depth research, it is revealed that the oncogenic roles of Beclin-1 in certain types of cancers could be attributed to its autophagic and non-autophagic functions. Moreover, Beclin-1 also participates in the development of resistance to cancer therapeutics and contributes to CSCs maintenance. Therefore, in this review, we provide an update on the controversial roles of Beclin-1 in tumorigenesis, unraveling the complexity of Beclin-1 in the context of diverse tumor subtypes. Additionally, various transcriptional, post-transcriptional, and post-translational regulations of Beclin-1 may contribute to its discrepant expression levels in different cancers, which can help us better understand how Beclin-1 is involved in tumorigenesis. However, more investigations are still required to elucidate specific molecular mechanisms in different cancer subtypes, which will be beneficial in defining the precise role of Beclin-1 in

mediating tumor progression and developing corresponding strategies to improve therapeutic outcomes.

To date, Beclin-1 has been considered an attractive therapeutic target to regulate autophagic activity for the treatment of cancer. Tat-Beclin-1 is the first described autophagy-inducing peptide that shows its therapeutic potential in cancer. Further efforts result in the discovery of other two Beclin-1-targeting peptides, namely Tat-SP4 and i7-01s-31, which could suppress cell proliferation through binding to Beclin-1 to promote autophagy initiation. Interestingly, given the positive role of Beclin-1 in dormant tumor cells, a Beclin-1-inhibiting peptide Tat-D3S2 has been designed to prevent the Beclin-1−DIRAS3 interaction in ovarian cancer cells, thus inhibiting pro-survival autophagy induced by starvation. Moreover, several small-molecule Beclin-1 activators, such as AG-690/12088124 and piceatannol, were discovered as anticancer agents, which could encourage the discovery of Beclin-1-targeting small-molecule drugs. Furthermore, targeting Beclin-1 interactomes to alter their protein−protein interaction, such as Bcl-2 and ATG14L, provide more opportunities to regulate Beclin-1 functions for therapeutic purposes[304−306]. Meanwhile, biomedical engineering-based products, such as tumor acidity-responsive TpB protein and OVV-BECN1, also exhibited adequate tumor-suppressive activity, which may provide promising opportunities for developing Beclin-1-targeting approaches. Nevertheless, there are still several issues that need to be addressed. Firstly, despite recent advances in the *in silico* protein-structure-prediction algorithm, the complexities of intrinsic disordered Beclin-1 N-terminal structure are not yet fully deciphered, which still poses a substantial challenge for discovery and optimization of Beclin-1-targeting small-molecule compounds. Regulating Beclin-1's functions by affecting its interactomes has emerged as a promising alternative. However, given a plethora of Beclin-1 interactomes, further investigation is required to deepen our understanding of the molecular mechanisms by which these interactomes context-dependently participate in the progression of specific tumors. Finally, the dual roles of Beclin-1-mediated autophagy in some contexts add an additional layer of complexity to the development of Beclin-1-targeting therapeutic strategies, which needs to be considered before these strategies are translated from bench to bedside.

In summary, this review provides an overview of the structure, functions, regulations, and controversial roles of Beclin-1, elucidating its potential mechanisms in oncology. Notably, the currently available targeted Beclin-1-regulating strategies are summarized, which will drive forward the search for Belcin-1-targeted therapy for cancer patients in the future.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant Nos. 22177084 and 82173666), Sichuan Science and Technology Program (Grant No. 2022YFQ0054, China), and the Open Research Fund of Chengdu University of Traditional Chinese Medicine State Key Laboratory of Characteristic Chinese Medicine Resources in Southwest China. We also thank some materials in the graphical abstract and figures that are produced by BioRender (https://biorender.com).

Author contributions

Bo Liu: Project administration, Supervision, Funding acquisition. Gu He: Conceptualization, Methodology, Supervision. Jing Ye: Data curation, Writing-Original Draft, Visualization. Jin Zhang: Writing-Review & Editing, Visualization. Yanghui Zhu: Data Curation. Lian Wang: Visualization. Xian Jiang: Supervision.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Singh SS, Vats S, Chia AY, Tan TZ, Deng S, Ong MS, et al. Dual role of autophagy in hallmarks of cancer. *Oncogene* 2018;**37**:1142−58.
2. Yun CW, Jeon J, Go G, Lee JH, Lee SH. The dual role of autophagy in cancer development and a therapeutic strategy for cancer by targeting autophagy. *Int J Mol Sci* 2020;**22**:179.
3. Zhang L, Zhu Y, Zhang J, Zhang L, Chen L. Inhibiting cytoprotective autophagy in cancer therapy: an update on pharmacological small-molecule compounds. *Front Pharmacol* 2022;**13**:966012.
4. Peng F, Liao M, Qin R, Zhu S, Peng C, Fu L, et al. Regulated cell death (RCD) in cancer: key pathways and targeted therapies. *Signal Transduct Targeted Ther* 2022;**7**:286.
5. Das CK, Mandal M, Kögel D. Pro-survival autophagy and cancer cell resistance to therapy. *Cancer Metastasis Rev* 2018;**37**:749−66.
6. Folkerts H, Hilgendorf S, Vellenga E, Bremer E, Wiersma VR. The multifaceted role of autophagy in cancer and the microenvironment. *Med Res Rev* 2019;**39**:517−60.
7. Vitto VAM, Bianchin S, Zolondick AA, Pellielo G, Rimessi A, Chianese D, et al. Molecular mechanisms of autophagy in cancer development, progression, and therapy. *Biomedicines* 2022;**10**:1596.
8. Anderson CM, Macleod KF. Autophagy and cancer cell metabolism. *Int Rev Cell Mol Biol* 2019;**347**:145−90.
9. Zhang J, Wang G, Zhou Y, Chen Y, Ouyang L, Liu B. Mechanisms of autophagy and relevant small-molecule compounds for targeted cancer therapy. *Cell Mol Life Sci* 2018;**75**:1803−26.
10. Wu J, Ye J, Xie Q, Liu B, Liu M. Targeting regulated cell death with pharmacological small molecules: an update on autophagy-dependent cell death, ferroptosis, and necroptosis in cancer. *J Med Chem* 2022;**65**:2989−3001.
11. Nakatogawa H. Mechanisms governing autophagosome biogenesis. *Nat Rev Mol Cell Biol* 2020;**21**:439−58.
12. Shibutani ST, Yoshimori T. A current perspective of autophagosome biogenesis. *Cell Res* 2014;**24**:58−68.
13. Zou L, Liao M, Zhen Y, Zhu S, Chen X, Zhang J, et al. Autophagy and beyond: unraveling the complexity of UNC-51-like kinase 1 (ULK1) from biological functions to therapeutic implications. *Acta Pharm Sin B* 2022;**12**:3743−82.
14. Zhang K, Zhu S, Li J, Jiang T, Feng L, Pei J, et al. Targeting autophagy using small-molecule compounds to improve potential therapy of Parkinson's disease. *Acta Pharm Sin B* 2021;**11**:3015−34.
15. Kametaka S, Okano T, Ohsumi M, Ohsumi Y. Apg14p and Apg6/Vps30p form a protein complex essential for autophagy in the yeast, *Saccharomyces cerevisiae*. *J Biol Chem* 1998;**273**:22284−91.
16. Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, et al. Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J Virol* 1998;**72**:8586−96.
17. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 1999;**402**:672−6.
18. Kihara A, Kabeya Y, Ohsumi Y, Yoshimori T. Beclin-phosphatidylinositol 3-kinase complex functions at the *trans*-Golgi network. *EMBO Rep* 2001;**2**:330−5.
19. Furuya N, Yu J, Byfield M, Pattingre S, Levine B. The evolutionarily conserved domain of Beclin 1 is required for Vps34 binding, autophagy and tumor suppressor function. *Autophagy* 2005;**1**:46−52.
20. Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, et al. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* 2006;**8**:688−99.

21. Itakura E, Kishi C, Inoue K, Mizushima N. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell* 2008;**19**:5360−72.
22. Liang C, Lee JS, Inn KS, Gack MU, Li Q, Roberts EA, et al. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat Cell Biol* 2008;**10**:776−87.
23. Gao X, Zacharek A, Salkowski A, Grignon DJ, Sakr W, Porter AT, et al. Loss of heterozygosity of the BRCA1 and other loci on chromosome 17q in human prostate cancer. *Cancer Res* 1995;**55**:1002−5.
24. Kundson AG Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A* 1971;**68**:820−3.
25. Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest* 2003;**112**:1809−20.
26. Lin HX, Qiu HJ, Zeng F, Rao HL, Yang GF, Kung HF, et al. Decreased expression of Beclin 1 correlates closely with Bcl-xL expression and poor prognosis of ovarian carcinoma. *PLoS One* 2013;**8**:e60516.
27. Dong M, Wan XB, Yuan ZY, Wei L, Fan XJ, Wang TT, et al. Low expression of Beclin 1 and elevated expression of HIF-1α refine distant metastasis risk and predict poor prognosis of ER-positive, HER2-negative breast cancer. *Med Oncol* 2013;**30**:355.
28. Tang H, Sebti S, Titone R, Zhou Y, Isidoro C, Ross TS, et al. Decreased *BECN1* mRNA expression in human breast cancer is associated with estrogen receptor-negative subtypes and poor prognosis. *EBioMedicine* 2015;**2**:255−63.
29. Baspinar S, Bircan S, Orhan H, Kapucuoglu N, Bozkurt KK. The relation of beclin 1 and bcl-2 expressions in high grade prostatic intraepithelial neoplasia and prostate adenocarcinoma: a tissue microarray study. *Pathol Res Pract* 2014;**210**:412−8.
30. Zheng HC, Zhao S, Xue H, Zhao EH, Jiang HM, Hao CL. The roles of Beclin 1 expression in gastric cancer: a marker for carcinogenesis, aggressive behaviors and favorable prognosis, and a target of gene therapy. *Front Oncol* 2020;**10**:613679.
31. Hu Z, Zhong Z, Huang S, Wen H, Chen X, Chu H, et al. Decreased expression of Beclin-1 is significantly associated with a poor prognosis in oral tongue squamous cell carcinoma. *Mol Med Rep* 2016;**14**:1567−73.
32. Sun H, Yu J, Wen Z, Wang M, Chen W. Decreased expression of Beclin-1 in patients with hepatocellular carcinoma. *J BUON* 2019;**24**:634−41.
33. Wang ZH, Xu L, Wang Y, Cao MQ, Li L, Bai T. Clinicopathologic correlations between human papillomavirus 16 infection and Beclin 1 expression in human cervical cancer. *Int J Gynecol Pathol* 2011;**30**:400−6.
34. Wang TT, Cao QH, Chen MY, Xia Q, Fan XJ, Ma XK, et al. Beclin 1 deficiency correlated with lymph node metastasis, predicts a distinct outcome in intrahepatic and extrahepatic cholangiocarcinoma. *PLoS One* 2013;**8**:e80317.
35. Chen C, Ma Q, Ma X, Liu Z, Liu X. Association of elevated HIF-2α levels with low Beclin 1 expression and poor prognosis in patients with chondrosarcoma. *Ann Surg Oncol* 2011;**18**:2364−72.
36. Miracco C, Cosci E, Oliveri G, Luzi P, Pacenti L, Monciatti I, et al. Protein and mRNA expression of autophagy gene Beclin 1 in human brain tumours. *Int J Oncol* 2007;**30**:429−36.
37. Pirtoli L, Cevenini G, Tini P, Vannini M, Oliveri G, Marsili S, et al. The prognostic role of Beclin 1 protein expression in high-grade gliomas. *Autophagy* 2009;**5**:930−6.
38. Miracco C, Cevenini G, Franchi A, Luzi P, Cosci E, Mourmouras V, et al. Beclin 1 and LC3 autophagic gene expression in cutaneous melanocytic lesions. *Hum Pathol* 2010;**41**:503−12.
39. Radwan SM, Hamdy NM, Hegab HM, El-Mesallamy HO. Beclin-1 and hypoxia-inducible factor-1α genes expression: potential biomarkers in acute leukemia patients. *Cancer Biomarkers* 2016;**16**:619−26.
40. Wang J, Pan XL, Ding LJ, Liu DY, Da-Peng L, Jin T. Aberrant expression of Beclin-1 and LC3 correlates with poor prognosis of human hypopharyngeal squamous cell carcinoma. *PLoS One* 2013;**8**:e69038.
41. Wechman SL, Pradhan AK, DeSalle R, Das SK, Emdad L, Sarkar D, et al. New insights into Beclin-1: evolution and pan-malignancy inhibitor activity. *Adv Cancer Res* 2018;**137**:77−114.
42. Koukourakis MI, Giatromanolaki A, Sivridis E, Pitiakoudis M, Gatter KC, Harris AL. Beclin 1 over- and underexpression in colorectal cancer: distinct patterns relate to prognosis and tumour hypoxia. *Br J Cancer* 2010;**103**:1209−14.
43. Gong C, Bauvy C, Tonelli G, Yue W, Deloménie C, Nicolas V, et al. Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. *Oncogene* 2013;**32**:2261−72. 72e.1-2272.
44. Ying H, Qu D, Liu C, Ying T, Lv J, Jin S, et al. Chemoresistance is associated with Beclin-1 and PTEN expression in epithelial ovarian cancers. *Oncol Lett* 2015;**9**:1759−63.
45. Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L, et al. Identification of a candidate therapeutic autophagy-inducing peptide. *Nature* 2013;**494**:201−6.
46. Vega-Rubín-de-Celis S, Zou Z, Fernández Á F, Ci B, Kim M, Xiao G, et al. Increased autophagy blocks HER2-mediated breast tumorigenesis. *Proc Natl Acad Sci U S A* 2018;**115**:4176−81.
47. Kang R, Zeh HJ, Lotze MT, Tang D. The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ* 2011;**18**:571−80.
48. Menon MB, Dhamija S. Beclin 1 phosphorylation—at the center of autophagy regulation. *Front Cell Dev Biol* 2018;**6**:137.
49. Mei Y, Ramanathan A, Glover K, Stanley C, Sanishvili R, Chakravarthy S, et al. Conformational flexibility enables the function of a BECN1 region essential for starvation-mediated autophagy. *Biochemistry* 2016;**55**:1945−58.
50. Li X, He L, Che KH, Funderburk SF, Pan L, Pan N, et al. Imperfect interface of Beclin1 coiled-coil domain regulates homodimer and heterodimer formation with Atg14L and UVRAG. *Nat Commun* 2012;**3**:662.
51. Sun Q, Fan W, Chen K, Ding X, Chen S, Zhong Q. Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* 2008;**105**:19211−6.
52. Maiuri MC, Le Toumelin G, Criollo A, Rain JC, Gautier F, Juin P, et al. Functional and physical interaction between Bcl-X_L and a BH3-like domain in Beclin-1. *EMBO J* 2007;**26**:2527−39.
53. Walensky LD. BCL-2 in the crosshairs: tipping the balance of life and death. *Cell Death Differ* 2006;**13**:1339−50.
54. Robert G, Gastaldi C, Puissant A, Hamouda A, Jacquel A, Dufies M, et al. The anti-apoptotic Bcl-B protein inhibits BECN1-dependent autophagic cell death. *Autophagy* 2012;**8**:637−49.
55. Chang C, Young LN, Morris KL, von Bülow S, Schöneberg J, Yamamoto-Imoto H, et al. Bidirectional control of autophagy by BECN1 BARA domain dynamics. *Mol Cell* 2019;**73**:339−353.e6.
56. Decuypere JP, Parys JB, Bultynck G. Regulation of the autophagic bcl-2/beclin 1 interaction. *Cells* 2012;**1**:284−312.
57. Strappazzon F, Vietri-Rudan M, Campello S, Nazio F, Florenzano F, Fimia GM, et al. Mitochondrial BCL-2 inhibits AMBRA1-induced autophagy. *EMBO J* 2011;**30**:1195−208.
58. Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, et al. Ambra1 regulates autophagy and development of the nervous system. *Nature* 2007;**447**:1121−5.
59. Morris DH, Yip CK, Shi Y, Chait BT, Wang QJ. Beclin 1−VPS34 complex architecture: understanding the nuts and bolts of therapeutic targets. *Front Biol* 2015;**10**:398−426.
60. Takahashi Y, Coppola D, Matsushita N, Cualing HD, Sun M, Sato Y, et al. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* 2007;**9**:1142−51.
61. Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P, et al. Endogenous HMGB1 regulates autophagy. *J Cell Biol* 2010;**190**:881−92.

62. Zhu Q, Song J, Chen JY, Yuan Z, Liu L, Xie LM, et al. Corynoxine B targets at HMGB1/2 to enhance autophagy for α-synuclein clearance in fly and rodent models of Parkinson's disease. *Acta Pharm Sin B* 2023;**13**:2701−14.
63. Lu Z, Baquero MT, Yang H, Yang M, Reger AS, Kim C, et al. DIRAS3 regulates the autophagosome initiation complex in dormant ovarian cancer cells. *Autophagy* 2014;**10**:1071−92.
64. Jounai N, Kobiyama K, Shiina M, Ogata K, Ishii KJ, Takeshita F. NLRP4 negatively regulates autophagic processes through an association with beclin1. *J Immunol* 2011;**186**:1646−55.
65. Zou J, Yue F, Jiang X, Li W, Yi J, Liu L. Mitochondrion-associated protein LRPPRC suppresses the initiation of basal levels of autophagy *via* enhancing Bcl-2 stability. *Biochem J* 2013;**454**:447−57.
66. Huang W, Choi W, Hu W, Mi N, Guo Q, Ma M, et al. Crystal structure and biochemical analyses reveal Beclin 1 as a novel membrane binding protein. *Cell Res* 2012;**22**:473−89.
67. Rostislavleva K, Soler N, Ohashi Y, Zhang L, Pardon E, Burke JE, et al. Structure and flexibility of the endosomal Vps34 complex reveals the basis of its function on membranes. *Sci* 2015;**350**:aac7365.
68. Baskaran S, Carlson LA, Stjepanovic G, Young LN, Kim DJ, Grob P, et al. Architecture and dynamics of the autophagic phosphatidylinositol 3-kinase complex. *Elife* 2014;**3**:e05115.
69. Anderson KE, Boyle KB, Davidson K, Chessa TA, Kulkarni S, Jarvis GE, et al. CD18-dependent activation of the neutrophil NADPH oxidase during phagocytosis of *Escherichia coli* or *Staphylococcus aureus* is regulated by class III but not class I or II PI3Ks. *Blood* 2008;**112**:5202−11.
70. Brier LW, Ge L, Stjepanovic G, Thelen AM, Hurley JH, Schekman R. Regulation of LC3 lipidation by the autophagy-specific class III phosphatidylinositol-3 kinase complex. *Mol Biol Cell* 2019;**30**:1098−107.
71. Caux M, Chicanne G, Severin S. Class III PI3K biology. *Curr Top Microbiol Immunol* 2022;**436**:69−93.
72. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. *Cell* 2019;**176**:11−42.
73. Foerster EG, Mukherjee T, Cabral-Fernandes L, Rocha JDB, Girardin SE, Philpott DJ. How autophagy controls the intestinal epithelial barrier. *Autophagy* 2022;**18**:86−103.
74. Itakura E, Mizushima N. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy* 2010;**6**:764−76.
75. Zachari M, Ganley IG. The mammalian ULK1 complex and autophagy initiation. *Essays Biochem* 2017;**61**:585−96.
76. Hsu P, Shi Y. Regulation of autophagy by mitochondrial phospholipids in health and diseases. *Biochim Biophys Acta Mol Cell Biol Lipids* 2017;**1862**:114−29.
77. Russell RC, Tian Y, Yuan H, Park HW, Chang YY, Kim J, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol* 2013;**15**:741−50.
78. Zhang W, Xu C, Sun J, Shen HM, Wang J, Yang C. Impairment of the autophagy−lysosomal pathway in Alzheimer's diseases: pathogenic mechanisms and therapeutic potential. *Acta Pharm Sin B* 2022;**12**:1019−40.
79. Ma M, Liu JJ, Li Y, Huang Y, Ta N, Chen Y, et al. Cryo-EM structure and biochemical analysis reveal the basis of the functional difference between human PI3KC3-C1 and -C2. *Cell Res* 2017;**27**:989−1001.
80. Nishimura T, Tooze SA. Emerging roles of ATG proteins and membrane lipids in autophagosome formation. *Cell discovery* 2020;**6**:32.
81. Vicinanza M, Korolchuk VI, Ashkenazi A, Puri C, Menzies FM, Clarke JH, et al. PI(5)P regulates autophagosome biogenesis. *Mol Cell* 2015;**57**:219−34.
82. Wu MY, Wang EJ, Feng D, Li M, Ye RD, Lu JH. Pharmacological insights into autophagy modulation in autoimmune diseases. *Acta Pharm Sin B* 2021;**11**:3364−78.
83. Dooley HC, Razi M, Polson HE, Girardin SE, Wilson MI, Tooze SA. WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12-5-16L1. *Mol Cell* 2014;**55**:238−52.
84. Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 2011;**27**:107−32.
85. Dooley HC, Wilson MI, Tooze SA. WIPI2B links PtdIns3P to LC3 lipidation through binding ATG16L1. *Autophagy* 2015;**11**:190−1.
86. Melia TJ, Lystad AH, Simonsen A. Autophagosome biogenesis: from membrane growth to closure. *J Cell Biol* 2020;**219**:e202002085.
87. Alemu EA, Lamark T, Torgersen KM, Birgisdottir AB, Larsen KB, Jain A, et al. ATG8 family proteins act as scaffolds for assembly of the ULK complex: sequence requirements for LC3-interacting region (LIR) motifs. *J Biol Chem* 2012;**287**:39275−90.
88. Mizushima N. A brief history of autophagy from cell biology to physiology and disease. *Nat Cell Biol* 2018;**20**:521−7.
89. McKnight NC, Zhong Y, Wold MS, Gong S, Phillips GR, Dou Z, et al. Beclin 1 is required for neuron viability and regulates endosome pathways *via* the UVRAG−VPS34 complex. *PLoS Genet* 2014;**10**:e1004626.
90. Thoresen SB, Pedersen NM, Liestøl K, Stenmark H. A phosphatidylinositol 3-kinase class III sub-complex containing VPS15, VPS34, Beclin 1, UVRAG and BIF-1 regulates cytokinesis and degradative endocytic traffic. *Exp Cell Res* 2010;**316**:3368−78.
91. Law F, Rocheleau CE. Vps34 and the armus/TBC-2 Rab GAPs: putting the brakes on the endosomal Rab5 and Rab7 GTPases. *Cell Logist* 2017;**7**:e1403530.
92. Backer JM. The intricate regulation and complex functions of the class III phosphoinositide 3-kinase Vps34. *Biochem J* 2016;**473**:2251−71.
93. Bonifacino JS, Hurley JH. Retromer. *Curr Opin Cell Biol* 2008;**20**:427−36.
94. Henne WM, Buchkovich NJ, Emr SD. The ESCRT pathway. *Dev Cell* 2011;**21**:77−91.
95. Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, et al. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat Cell Biol* 2009;**11**:385−96.
96. Liu J, Li M, Li L, Chen S, Wang X. Ubiquitination of the PI3-kinase VPS-34 promotes VPS-34 stability and phagosome maturation. *J Cell Biol* 2018;**217**:347−60.
97. Wirawan E, Vande Walle L, Kersse K, Cornelis S, Claerhout S, Vanoverberghe I, et al. Caspase-mediated cleavage of Beclin-1 inactivates Beclin-1-induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. *Cell Death Dis* 2010;**1**:e18.
98. Li H, Wang P, Yu J, Zhang L. Cleaving Beclin 1 to suppress autophagy in chemotherapy-induced apoptosis. *Autophagy* 2011;**7**:1239−41.
99. Zhu Y, Zhao L, Liu L, Gao P, Tian W, Wang X, et al. Beclin 1 cleavage by caspase-3 inactivates autophagy and promotes apoptosis. *Protein Cell* 2010;**1**:468−77.
100. Siddiqui MA, Mukherjee S, Manivannan P, Malathi K. RNase L cleavage products promote switch from autophagy to apoptosis by caspase-mediated cleavage of Beclin-1. *Int J Mol Sci* 2015;**16**:17611−36.
101. Song X, Zhu S, Chen P, Hou W, Wen Q, Liu J, et al. AMPK-mediated BECN1 phosphorylation promotes ferroptosis by directly blocking system X_c^- activity. *Curr Biol* 2018;**28**:2388−2399.e5.
102. Seo J, Seong D, Nam YW, Hwang CH, Lee SR, Lee CS, et al. Beclin 1 functions as a negative modulator of MLKL oligomerisation by integrating into the necrosome complex. *Cell Death Differ* 2020;**27**:3065−81.
103. Park JM, Tougeron D, Huang S, Okamoto K, Sinicrope FA. Beclin 1 and UVRAG confer protection from radiation-induced DNA damage and maintain centrosome stability in colorectal cancer cells. *PLoS One* 2014;**9**:e100819.
104. Xu F, Fang Y, Yan L, Xu L, Zhang S, Cao Y, et al. Nuclear localization of Beclin 1 promotes radiation-induced DNA damage repair independent of autophagy. *Sci Rep* 2017;**7**:45385.

105. Wijshake T, Zou Z, Chen B, Zhong L, Xiao G, Xie Y, et al. Tumor-suppressor function of Beclin 1 in breast cancer cells requires E-cadherin. *Proc Natl Acad Sci U S A* 2021;**118**:e2020478118.
106. Lee SJ, Kim HP, Jin Y, Choi AM, Ryter SW. Beclin 1 deficiency is associated with increased hypoxia-induced angiogenesis. *Autophagy* 2011;**7**:829−39.
107. Sun Y, Liu JH, Sui YX, Jin L, Yang Y, Lin SM, et al. Beclin1 overexpression inhibits proliferation, invasion and migration of CaSki cervical cancer cells. *Asian Pac J Cancer Prev APJCP* 2011; **12**:1269−73.
108. Su YL, Kortylewski M. Beclin-1 as a neutrophil-specific immune checkpoint. *J Clin Invest* 2019;**129**:5079−81.
109. Tan P, He L, Xing C, Mao J, Yu X, Zhu M, et al. Myeloid loss of *Beclin 1* promotes PD-L1^{hi} precursor B cell lymphoma development. *J Clin Invest* 2019;**129**:5261−77.
110. Copetti T, Demarchi F, Schneider C. p65/RelA binds and activates the beclin 1 promoter. *Autophagy* 2009;**5**:858−9.
111. Copetti T, Bertoli C, Dalla E, Demarchi F, Schneider C. p65/RelA modulates *BECN1* transcription and autophagy. *Mol Cell Biol* 2009; **29**:2594−608.
112. Lin F, Ghislat G, Luo S, Renna M, Siddiqi F, Rubinsztein DC. XIAP and cIAP1 amplifications induce Beclin 1-dependent autophagy through NFκB activation. *Hum Mol Genet* 2015;**24**:2899−913.
113. Han T, Guo M, Gan M, Yu B, Tian X, Wang JB. TRIM59 regulates autophagy through modulating both the transcription and the ubiquitination of BECN1. *Autophagy* 2018;**14**:2035−48.
114. Zhu W, Swaminathan G, Plowey ED. GA binding protein augments autophagy *via* transcriptional activation of *BECN1−PIK3C3* complex genes. *Autophagy* 2014;**10**:1622−36.
115. Hamurcu Z, Delibaşı N, Nalbantoglu U, Sener EF, Nurdinov N, Tascı B, et al. FOXM1 plays a role in autophagy by transcriptionally regulating Beclin-1 and LC3 genes in human triple-negative breast cancer cells. *J Mol Med (Berl)* 2019;**97**:491−508.
116. Jia J, Zhang HB, Shi Q, Yang C, Ma JB, Jin B, et al. KLF5 down-regulation desensitizes castration-resistant prostate cancer cells to docetaxel by increasing BECN1 expression and inducing cell autophagy. *Theranostics* 2019;**9**:5464−77.
117. Li Z, Chen B, Wu Y, Jin F, Xia Y, Liu X. Genetic and epigenetic silencing of the beclin 1 gene in sporadic breast tumors. *BMC Cancer* 2010;**10**:98.
118. Park SE, Yi HJ, Suh N, Park YY, Koh JY, Jeong SY, et al. Inhibition of EHMT2/G9a epigenetically increases the transcription of Beclin-1 *via* an increase in ROS and activation of NF-κB. *Oncotarget* 2016;**7**: 39796−808.
119. Zhu H, Wu H, Liu X, Li B, Chen Y, Ren X, et al. Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. *Autophagy* 2009;**5**:816−23.
120. Korkmaz G, le Sage C, Tekirdag KA, Agami R, Gozuacik D. miR-376b controls starvation and mTOR inhibition-related autophagy by targeting ATG4C and BECN1. *Autophagy* 2012;**8**:165−76.
121. Xu R, Liu S, Chen H, Lao L. MicroRNA-30a downregulation contributes to chemoresistance of osteosarcoma cells through activating Beclin-1-mediated autophagy. *Oncol Rep* 2016;**35**:1757−63.
122. Pradhan AK, Talukdar S, Bhoopathi P, Shen XN, Emdad L, Das SK, et al. *mda-7/IL-24* mediates cancer cell-specific death *via* regulation of miR-221 and the Beclin-1 axis. *Cancer Res* 2017;**77**:949−59.
123. Zhang X, Shi H, Lin S, Ba M, Cui S. MicroRNA-216a enhances the radiosensitivity of pancreatic cancer cells by inhibiting beclin-1-mediated autophagy. *Oncol Rep* 2015;**34**:1557−64.
124. Tan S, Shi H, Ba M, Lin S, Tang H, Zeng X, et al. miR-409-3p sensitizes colon cancer cells to oxaliplatin by inhibiting Beclin-1-mediated autophagy. *Int J Mol Med* 2016;**37**:1030−8.
125. Li M, Chen XM, Wang DM, Gan L, Qiao Y. Effects of miR-26a on the expression of Beclin 1 in retinoblastoma cells. *Genet Mol Res* 2016;**15**:15028193. gmr.
126. Hou W, Song L, Zhao Y, Liu Q, Zhang S. Inhibition of Beclin-1-mediated autophagy by microRNA-17-5p enhanced the radiosensitivity of glioma cells. *Oncol Res* 2017;**25**:43−53.
127. Zhang F, Wang B, Long H, Yu J, Li F, Hou H, et al. Decreased miR-124-3p expression prompted breast cancer cell progression mainly by targeting Beclin-1. *Clin Lab* 2016;**62**:1139−45.
128. Huang T, Wan X, Alvarez AA, James CD, Song X, Yang Y, et al. MIR93 (microRNA-93) regulates tumorigenicity and therapy response of glioblastoma by targeting autophagy. *Autophagy* 2019;**15**:1100−11.
129. Yu JL, Gao X. MicroRNA 1301 inhibits cisplatin resistance in human ovarian cancer cells by regulating EMT and autophagy. *Eur Rev Med Pharmacol Sci* 2020;**24**:1688−96.
130. Zhang HH, Huang ZX, Zhong SQ, Fei KL, Cao YH. miR-21 inhibits autophagy and promotes malignant development in the bladder cancer T24 cell line. *Int J Oncol* 2020;**56**:986−98.
131. Luo M, Wu L, Zhang K, Wang H, Wu S, O'Connell D, et al. miR-216b enhances the efficacy of vemurafenib by targeting Beclin-1, UVRAG and ATG5 in melanoma. *Cell Signal* 2018;**42**:30−43.
132. Chen Y, Li Z, Chen X, Zhang S. Long non-coding RNAs: from disease code to drug role. *Acta Pharm Sin B* 2021;**11**:340−54.
133. Han S, Chen X, Huang L. The tumor therapeutic potential of long non-coding RNA delivery and targeting. *Acta Pharm Sin B* 2023;**13**: 1371−82.
134. Wang J, Xie S, Yang J, Xiong H, Jia Y, Zhou Y, et al. The long noncoding RNA H19 promotes tamoxifen resistance in breast cancer *via* autophagy. *J Hematol Oncol* 2019;**12**:81.
135. Zhang L, Wang Y, Xia S, Yang L, Wu D, Zhou Y, et al. Long noncoding RNA PANDAR inhibits the development of lung cancer by regulating autophagy and apoptosis pathways. *J Cancer* 2020;**11**:4783−90.
136. Wang Y, Li Z, Xu S, Li W, Chen M, Jiang M, et al. LncRNA FIRRE functions as a tumor promoter by interaction with PTBP1 to stabilize *BECN1* mRNA and facilitate autophagy. *Cell Death Dis* 2022;**13**:98.
137. Li D, Li C, Chen Y, Teng L, Cao Y, Wang W, et al. LncRNA HOTAIR induces sunitinib resistance in renal cancer by acting as a competing endogenous RNA to regulate autophagy of renal cells. *Cancer Cell Int* 2020;**20**:338.
138. Chen L, Han X, Hu Z, Chen L. The PVT1/miR-216b/Beclin-1 regulates cisplatin sensitivity of NSCLC cells *via* modulating autophagy and apoptosis. *Cancer Chemother Pharmacol* 2019;**83**:921−31.
139. Baralle FE, Giudice J. Alternative splicing as a regulator of development and tissue identity. *Nat Rev Mol Cell Biol* 2017;**18**:437−51.
140. Li D, Yu W, Lai M. Towards understandings of serine/arginine-rich splicing factors. *Acta Pharm Sin B* 2023;**13**:3181−207.
141. Cheng B, Xu A, Qiao M, Wu Q, Wang W, Mei Y, et al. BECN1s, a short splice variant of BECN1, functions in mitophagy. *Autophagy* 2015;**11**:2048−56.
142. Maheshwari C, Vidoni C, Titone R, Castiglioni A, Lora C, Follo C, et al. Isolation, characterization, and autophagy function of BECN1-splicing isoforms in cancer cells. *Biomolecules* 2022;**12**:1069.
143. Rabanal-Ruiz Y, Otten EG, Korolchuk VI. mTORC1 as the main gateway to autophagy. *Essays Biochem* 2017;**61**:565−84.
144. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, et al. Nutrient-dependent mTORC1 association with the ULK1−Atg13−FIP200 complex required for autophagy. *Mol Biol Cell* 2009;**20**:1981−91.
145. Park JM, Seo M, Jung CH, Grunwald D, Stone M, Otto NM, et al. ULK1 phosphorylates Ser30 of BECN1 in association with ATG14 to stimulate autophagy induction. *Autophagy* 2018;**14**:584−97.
146. Qian X, Li X, Cai Q, Zhang C, Yu Q, Jiang Y, et al. Phosphoglycerate kinase 1 phosphorylates Beclin1 to induce autophagy. *Mol Cell* 2017; **65**:917−931.e6.
147. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 2011;**13**:132−41.
148. Zhang D, Wang W, Sun X, Xu D, Wang C, Zhang Q, et al. AMPK regulates autophagy by phosphorylating BECN1 at threonine 388. *Autophagy* 2016;**12**:1447−59.
149. Kim J, Kim YC, Fang C, Russell RC, Kim JH, Fan W, et al. Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell* 2013;**152**:290−303.

150. Wei Y, An Z, Zou Z, Sumpter R, Su M, Zang X, et al. The stress-responsive kinases MAPKAPK2/MAPKAPK3 activate starvation-induced autophagy through Beclin 1 phosphorylation. *Elife* 2015;**4**:e05289.
151. Fujiwara N, Usui T, Ohama T, Sato K. Regulation of Beclin 1 protein phosphorylation and autophagy by protein phosphatase 2A (PP2A) and death-associated protein kinase 3 (DAPK3). *J Biol Chem* 2016;**291**:10858−66.
152. Li X, Wu XQ, Deng R, Li DD, Tang J, Chen WD, et al. CaMKII-mediated Beclin 1 phosphorylation regulates autophagy that promotes degradation of Id and neuroblastoma cell differentiation. *Nat Commun* 2017;**8**:1159.
153. Zalckvar E, Berissi H, Eisenstein M, Kimchi A. Phosphorylation of Beclin 1 by DAP-kinase promotes autophagy by weakening its interactions with Bcl-2 and Bcl-XL. *Autophagy* 2009;**5**:720−2.
154. Zalckvar E, Berissi H, Mizrachy L, Idelchuk Y, Koren I, Eisenstein M, et al. DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. *EMBO Rep* 2009;**10**:285−92.
155. Shiloh R, Kimchi A. AMPK activates DAPK2 to promote autophagy. *Oncotarget* 2018;**9**:31570−1.
156. Gurkar AU, Chu K, Raj L, Bouley R, Lee SH, Kim YB, et al. Identification of ROCK1 kinase as a critical regulator of Beclin1-mediated autophagy during metabolic stress. *Nat Commun* 2013;**4**:2189.
157. Lee EF, Smith NA, Soares da Costa TP, Meftahi N, Yao S, Harris TJ, et al. Structural insights into BCL2 pro-survival protein interactions with the key autophagy regulator BECN1 following phosphorylation by STK4/MST1. *Autophagy* 2019;**15**:785−95.
158. Wang RC, Wei Y, An Z, Zou Z, Xiao G, Bhagat G, et al. Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. *Sci* 2012;**338**:956−9.
159. Sun T, Li X, Zhang P, Chen WD, Zhang HL, Li DD, et al. Acetylation of Beclin 1 inhibits autophagosome maturation and promotes tumour growth. *Nat Commun* 2015;**6**:7215.
160. Wei Y, Zou Z, Becker N, Anderson M, Sumpter R, Xiao G, et al. EGFR-mediated Beclin 1 phosphorylation in autophagy suppression, tumor progression, and tumor chemoresistance. *Cell* 2013;**154**:1269−84.
161. Yu C, Gorantla SP, Müller-Rudorf A, Müller TA, Kreutmair S, Albers C, et al. Phosphorylation of BECLIN-1 by BCR-ABL suppresses autophagy in chronic myeloid leukemia. *Haematologica* 2020;**105**:1285−93.
162. Hu F, Song D, Yan Y, Huang C, Shen C, Lan J, et al. IL-6 regulates autophagy and chemotherapy resistance by promoting BECN1 phosphorylation. *Nat Commun* 2021;**12**:3651.
163. Grabbe C, Husnjak K, Dikic I. The spatial and temporal organization of ubiquitin networks. *Nat Rev Mol Cell Biol* 2011;**12**:295−307.
164. Kaur S, Changotra H. The beclin 1 interactome: modification and roles in the pathology of autophagy-related disorders. *Biochimie* 2020;**175**:34−49.
165. Hill SM, Wrobel L, Rubinsztein DC. Post-translational modifications of Beclin 1 provide multiple strategies for autophagy regulation. *Cell Death Differ* 2019;**26**:617−29.
166. Platta HW, Abrahamsen H, Thoresen SB, Stenmark H. Nedd4-dependent lysine-11-linked polyubiquitination of the tumour suppressor Beclin 1. *Biochem J* 2012;**441**:399−406.
167. Tomaipitinca L, Petrungaro S, D'Acunzo P, Facchiano A, Dubey A, Rizza S, et al. c-FLIP regulates autophagy by interacting with Beclin-1 and influencing its stability. *Cell Death Dis* 2021;**12**:686.
168. Li X, Yang KB, Chen W, Mai J, Wu XQ, Sun T, et al. CUL3 (cullin 3)-mediated ubiquitination and degradation of BECN1 (beclin 1) inhibit autophagy and promote tumor progression. *Autophagy* 2021;**17**:4323−40.
169. Fusco C, Mandriani B, Di Rienzo M, Micale L, Malerba N, Cocciadiferro D, et al. TRIM50 regulates Beclin 1 proautophagic activity. *Biochim Biophys Acta Mol Cell Res* 2018;**1865**:908−19.
170. Shi CS, Kehrl JH. TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. *Sci Signal* 2010;**3**:ra42.
171. Shi CS, Kehrl JH. Traf6 and A20 differentially regulate TLR4-induced autophagy by affecting the ubiquitination of Beclin 1. *Autophagy* 2010;**6**:986−7.
172. Kim MJ, Min Y, Jeong SK, Son J, Kim JY, Lee JS, et al. USP15 negatively regulates lung cancer progression through the TRAF6−BECN1 signaling axis for autophagy induction. *Cell Death Dis* 2022;**13**:348.
173. Xia P, Wang S, Du Y, Zhao Z, Shi L, Sun L, et al. WASH inhibits autophagy through suppression of Beclin 1 ubiquitination. *EMBO J* 2013;**32**:2685−96.
174. Liu H, Ma Y, He HW, Wang JP, Jiang JD, Shao RG. SLC9A3R1 stimulates autophagy *via* BECN1 stabilization in breast cancer cells. *Autophagy* 2015;**11**:2323−34.
175. Song W, Zeng Z, Zhang Y, Li H, Cheng H, Wang J, et al. CircRNF144B/miR-342-3p/FBXL11 axis reduced autophagy and promoted the progression of ovarian cancer by increasing the ubiquitination of Beclin-1. *Cell Death Dis* 2022;**13**:857.
176. Xu D, Shan B, Sun H, Xiao J, Zhu K, Xie X, et al. USP14 regulates autophagy by suppressing K63 ubiquitination of Beclin 1. *Genes Dev* 2016;**30**:1718−30.
177. Min Y, Lee S, Kim MJ, Chun E, Lee KY. Ubiquitin-specific protease 14 negatively regulates toll-like receptor 4-mediated signaling and autophagy induction by inhibiting ubiquitination of TAK1-binding protein 2 and Beclin 1. *Front Immunol* 2017;**8**:1827.
178. Elgendy M, Ciro M, Abdel-Aziz AK, Belmonte G, Dal Zuffo R, Mercurio C, et al. Beclin 1 restrains tumorigenesis through Mcl-1 destabilization in an autophagy-independent reciprocal manner. *Nat Commun* 2014;**5**:5637.
179. Liu J, Xia H, Kim M, Xu L, Li Y, Zhang L, et al. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell* 2011;**147**:223−34.
180. Li J, Wang Y, Luo Y, Liu Y, Yi Y, Li J, et al. USP5−Beclin 1 axis overrides p53-dependent senescence and drives Kras-induced tumorigenicity. *Nat Commun* 2022;**13**:7799.
181. Mirzalieva O, Juncker M, Schwartzenburg J, Desai S. ISG15 and ISGylation in human diseases. *Cells* 2022;**11**:538.
182. Xu D, Zhang T, Xiao J, Zhu K, Wei R, Wu Z, et al. Modification of BECN1 by ISG15 plays a crucial role in autophagy regulation by type I IFN/interferon. *Autophagy* 2015;**11**:617−28.
183. Li C, Wang Y, Zheng H, Dong W, Lv H, Lin J, et al. Antiviral activity of ISG15 against classical swine fever virus replication in porcine alveolar macrophages *via* inhibition of autophagy by ISGylating BECN1. *Vet Res* 2020;**51**:22.
184. Sheng Z, Zhu J, Deng YN, Gao S, Liang S. SUMOylation modification-mediated cell death. *Open biology* 2021;**11**:210050.
185. Liu K, Guo C, Lao Y, Yang J, Chen F, Zhao Y, et al. A fine-tuning mechanism underlying self-control for autophagy: deSUMOylation of BECN1 by SENP3. *Autophagy* 2020;**16**:975−90.
186. Gil J, Ramírez-Torres A, Encarnación-Guevara S. Lysine acetylation and cancer: a proteomics perspective. *J Proteonomics* 2017;**150**:297−309.
187. Cho DH, Jo YK, Hwang JJ, Lee YM, Roh SA, Kim JC. Caspase-mediated cleavage of ATG6/Beclin-1 links apoptosis to autophagy in HeLa cells. *Cancer Lett* 2009;**274**:95−100.
188. Djavaheri-Mergny M, Maiuri MC, Kroemer G. Cross talk between apoptosis and autophagy by caspase-mediated cleavage of Beclin 1. *Oncogene* 2010;**29**:1717−9.
189. Luo S, Rubinsztein DC. Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-xL. *Cell Death Differ* 2010;**17**:268−77.
190. Li H, Wang P, Sun Q, Ding WX, Yin XM, Sobol RW, et al. Following cytochrome *c* release, autophagy is inhibited during chemotherapy-induced apoptosis by caspase 8-mediated cleavage of Beclin 1. *Cancer Res* 2011;**71**:3625−34.

191. Peng Y, Miao H, Wu S, Yang W, Zhang Y, Xie G, et al. ABHD5 interacts with BECN1 to regulate autophagy and tumorigenesis of colon cancer independent of PNPLA2. *Autophagy* 2016;**12**:2167−82.
192. Russo R, Berliocchi L, Adornetto A, Varano GP, Cavaliere F, Nucci C, et al. Calpain-mediated cleavage of Beclin-1 and autophagy deregulation following retinal ischemic injury *in vivo*. *Cell Death Dis* 2011;**2**:e144.
193. Nguyen HQ, Zada S, Lai TH, Pham TM, Hwang JS, Ahmed M, et al. Calpain-dependent Beclin1 cleavage stimulates senescence-associated cell death in HT22 hippocampal cells under the oxidative stress conditions. *Neurosci Lett* 2019;**701**:106−11.
194. Hu YJ, Zhong JT, Gong L, Zhang SC, Zhou SH. Autophagy-related Beclin 1 and head and neck cancers. *OncoTargets Ther* 2020;**13**:6213−27.
195. Jung YY, Lee YK, Koo JS. The potential of Beclin 1 as a therapeutic target for the treatment of breast cancer. *Expert Opin Ther Targets* 2016;**20**:167−78.
196. Russell SE, Hickey GI, Lowry WS, White P, Atkinson RJ. Allele loss from chromosome 17 in ovarian cancer. *Oncogene* 1990;**5**:1581−3.
197. Eccles DM, Russell SE, Haites NE, Atkinson R, Bell DW, Gruber L, et al. Early loss of heterozygosity on 17q in ovarian cancer. The abe ovarian cancer genetics group. *Oncogene* 1992;**7**:2069−72.
198. Cliby W, Ritland S, Hartmann L, Dodson M, Halling KC, Keeney G, et al. Human epithelial ovarian cancer allelotype. *Cancer Res* 1993;**53**:2393−8.
199. Tangir J, Muto MG, Berkowitz RS, Welch WR, Bell DA, Mok SC. A 400 kb novel deletion unit centromeric to the *BRCA1* gene in sporadic epithelial ovarian cancer. *Oncogene* 1996;**12**:735−40.
200. Salwa A, Ferraresi A, Chinthakindi M, Vallino L, Vidoni C, Dhanasekaran DN, et al. *BECN1* and *BRCA1* deficiency sensitizes ovarian cancer to platinum therapy and confers better prognosis. *Biomedicines* 2021;**9**:207.
201. Minamoto T, Nakayama K, Nakamura K, Katagiri H, Sultana R, Ishibashi T, et al. Loss of beclin 1 expression in ovarian cancer: a potential biomarker for predicting unfavorable outcomes. *Oncol Lett* 2018;**15**:1170−6.
202. Katagiri H, Nakayama K, Razia S, Nakamura K, Sato E, Ishibashi T, et al. Loss of autophagy-related protein Beclin 1 may define poor prognosis in ovarian clear cell carcinomas. *Int J Oncol* 2015;**47**:2037−44.
203. Lee JW, Jeong EG, Lee SH, Yoo NJ, Lee SH. Somatic mutations of BECN1, an autophagy-related gene, in human cancers. *APMIS* 2007;**115**:750−6.
204. Futreal PA, Söderkvist P, Marks JR, Iglehart JD, Cochran C, Barrett JC, et al. Detection of frequent allelic loss on proximal chromosome 17q in sporadic breast carcinoma using microsatellite length polymorphisms. *Cancer Res* 1992;**52**:2624−7.
205. Saito H, Inazawa J, Saito S, Kasumi F, Koi S, Sagae S, et al. Detailed deletion mapping of chromosome 17q in ovarian and breast cancers: 2-cM region on 17q21.3 often and commonly deleted in tumors. *Cancer Res* 1993;**53**:3382−5.
206. Choi J, Jung W, Koo JS. Expression of autophagy-related markers beclin-1, light chain 3A, light chain 3B and p62 according to the molecular subtype of breast cancer. *Histopathology* 2013;**62**:275−86.
207. Giatromanolaki A, Koukourakis MI, Georgiou I, Kouroupi M, Sivridis E. LC3A, LC3B and beclin-1 expression in gastric cancer. *Anticancer Res* 2018;**38**:6827−33.
208. Fei B, Ji F, Chen X, Liu Z, Li S, Mo Z, et al. Expression and clinical significance of Beclin-1 in gastric cancer tissues of various clinical stages. *Oncol Lett* 2016;**11**:2271−7.
209. Ahn CH, Jeong EG, Lee JW, Kim MS, Kim SH, Kim SS, et al. Expression of beclin-1, an autophagy-related protein, in gastric and colorectal cancers. *APMIS* 2007;**115**:1344−9.
210. Geng QR, Xu DZ, He LJ, Lu JB, Zhou ZW, Zhan YQ, et al. Beclin-1 expression is a significant predictor of survival in patients with lymph node-positive gastric cancer. *PLoS One* 2012;**7**:e45968.
211. Won KY, Kim GY, Lim SJ, Sung JY, Kim YW, Park YK, et al. Autophagy is related to the hedgehog signaling pathway in human gastric adenocarcinoma: prognostic significance of Beclin-1 and Gli2 expression in human gastric adenocarcinoma. *Pathol Res Pract* 2015;**211**:308−15.
212. Song MJ, Park S, Won KY. Expression of Beclin-1, an autophagy-related protein, is associated with tumoral FOXP3 expression and Tregs in gastric adenocarcinoma: the function of Beclin-1 expression as a favorable prognostic factor in gastric adenocarcinoma. *Pathol Res Pract* 2020;**216**:152927.
213. Masuda GO, Yashiro M, Kitayama K, Miki Y, Kasashima H, Kinoshita H, et al. Clinicopathological correlations of autophagy-related proteins LC3, Beclin 1 and p62 in gastric cancer. *Anticancer Res* 2016;**36**:129−36.
214. Zaanan A, Park JM, Tougeron D, Huang S, Wu TT, Foster NR, et al. Association of beclin 1 expression with response to neoadjuvant chemoradiation therapy in patients with locally advanced rectal carcinoma. *Int J Cancer* 2015;**137**:1498−502.
215. Yang Z, Ghoorun RA, Fan X, Wu P, Bai Y, Li J, et al. High expression of Beclin-1 predicts favorable prognosis for patients with colorectal cancer. *Clin Res Hepatol Gastroenterol* 2015;**39**:98−106.
216. Zhang MY, Gou WF, Zhao S, Mao XY, Zheng ZH, Takano Y, et al. Beclin 1 expression is closely linked to colorectal carcinogenesis and distant metastasis of colorectal carcinoma. *Int J Mol Sci* 2014;**15**:14372−85.
217. Li BX, Li CY, Peng RQ, Wu XJ, Wang HY, Wan DS, et al. The expression of beclin 1 is associated with favorable prognosis in stage IIIB colon cancers. *Autophagy* 2009;**5**:303−6.
218. Chen Z, Li Y, Zhang C, Yi H, Wu C, Wang J, et al. Downregulation of Beclin 1 and impairment of autophagy in a small population of colorectal cancer. *Dig Dis Sci* 2013;**58**:2887−94.
219. Barca I, Mignogna C, Novembre D, Ferragina F, Cristofaro MG. Immunohistochemical analysis of the Beclin-1 expression predicts the progression of oral squamous cell carcinoma. *Int J Environ Res Publ Health* 2021;**18**:11125.
220. Zhou W, Yue C, Deng J, Hu R, Xu J, Feng L, et al. Autophagic protein Beclin 1 serves as an independent positive prognostic biomarker for non-small cell lung cancer. *PLoS One* 2013;**8**:e80338.
221. Du H, Chen L, Luo F, Chen X, Li Y, Cheng Q. Beclin-1 expression is associated with prognosis in a Bcl-2-dependent manner in non-small cell lung cancer. *Oncol Lett* 2020;**20**:9.
222. Ding ZB, Shi YH, Zhou J, Qiu SJ, Xu Y, Dai Z, et al. Association of autophagy defect with a malignant phenotype and poor prognosis of hepatocellular carcinoma. *Cancer Res* 2008;**68**:9167−75.
223. Qin Z, Yu X, Lin M, Wu J, Ma S, Wang N. Prognostic and clinicopathological value of Beclin-1 expression in hepatocellular carcinoma: a meta-analysis. *World J Surg Oncol* 2018;**16**:170.
224. Al-Shenawy HA. Expression of Beclin-1, an autophagy-related marker, in chronic hepatitis and hepatocellular carcinoma and its relation with apoptotic markers. *APMIS* 2016;**124**:229−37.
225. Kuo KL, Chen CH, Chen HI, Chung YY, Chai CY. Higher expression of beclin 1 in human meningiomas is related to better clinical outcome and pathological grade. *APMIS* 2019;**127**:746−52.
226. Chen M, Li Q, Chen W, Bi J, Huang P. Diagnostic and prognostic value of Beclin 1 expression in melanoma: a meta-analysis. *Melanoma Res* 2021;**31**:541−9.
227. Broggi G, Ieni A, Russo D, Varricchio S, Puzzo L, Russo A, et al. The macro-autophagy-related protein Beclin-1 immunohistochemical expression correlates with tumor cell type and clinical behavior of uveal melanoma. *Front Oncol* 2020;**10**:589849.
228. Huang JJ, Zhu YJ, Lin TY, Jiang WQ, Huang HQ, Li ZM. Beclin 1 expression predicts favorable clinical outcome in patients with diffuse large B-cell lymphoma treated with R-CHOP. *Hum Pathol* 2011;**42**:1459−66.
229. Chen Y, Lu Y, Lu C, Zhang L. Beclin-1 expression is a predictor of clinical outcome in patients with esophageal squamous cell carcinoma and correlated to hypoxia-inducible factor (HIF)-1alpha expression. *Pathol Oncol Res* 2009;**15**:487−93.
230. Du H, Luo F, Shi M, Che J, Zhu L, Li H, et al. Beclin-1 is a promising prognostic biomarker in a specific esophageal squamous cell carcinoma population. *Pathol Oncol Res* 2021;**27**:594724.

231. Kim HS, Lee SH, Do SI, Lim SJ, Park YK, Kim YW. Clinicopathologic correlation of beclin-1 expression in pancreatic ductal adenocarcinoma. *Pathol Res Pract* 2011;**207**:247−52.
232. Wan XB, Fan XJ, Chen MY, Xiang J, Huang PY, Guo L, et al. Elevated Beclin 1 expression is correlated with HIF-1alpha in predicting poor prognosis of nasopharyngeal carcinoma. *Autophagy* 2010;**6**:395−404.
233. Li X, Xu H, Ma H. Beclin 1 is highly expressed in papillary thyroid carcinoma and correlates with lymph node metastasis. *Acta Chir Belg* 2013;**113**:175−81.
234. Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci U S A* 2003;**100**:15077−82.
235. Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* 2006;**10**:51−64.
236. Fernández Á F, Sebti S, Wei Y, Zou Z, Shi M, McMillan KL, et al. Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. *Nature* 2018;**558**:136−40.
237. Zhang MY, Wang LY, Zhao S, Guo XC, Xu YQ, Zheng ZH, et al. Effects of Beclin 1 overexpression on aggressive phenotypes of colon cancer cells. *Oncol Lett* 2019;**17**:2441−50.
238. Huang X, Qi Q, Hua X, Li X, Zhang W, Sun H, et al. Beclin 1, an autophagy-related gene, augments apoptosis in U87 glioblastoma cells. *Oncol Rep* 2014;**31**:1761−7.
239. Wang ZH, Xu L, Duan ZL, Zeng LQ, Yan NH, Peng ZL. Beclin 1-mediated macroautophagy involves regulation of caspase-9 expression in cervical cancer HeLa cells. *Gynecol Oncol* 2007;**107**:107−13.
240. Wang Y, Xie J, Wang H, Huang H, Xie P. Beclin-1 suppresses gastric cancer progression by promoting apoptosis and reducing cell migration. *Oncol Lett* 2017;**14**:6857−62.
241. Liu L, Zhao WM, Yang XH, Sun ZQ, Jin HZ, Lei C, et al. Effect of inhibiting Beclin-1 expression on autophagy, proliferation and apoptosis in colorectal cancer. *Oncol Lett* 2017;**14**:4319−24.
242. Wang W, Fan H, Zhou Y, Duan P, Zhao G, Wu G. Knockdown of autophagy-related gene *BECLIN1* promotes cell growth and inhibits apoptosis in the A549 human lung cancer cell line. *Mol Med Rep* 2013;**7**:1501−5.
243. Zhang J, Dong W. Lentiviral-mediated Beclin-1 overexpression inhibits cell proliferation and induces autophagy of human esophageal carcinoma Eca109 cell xenograft in nude mice. *Recent Pat Anti-Cancer Drug Discov* 2020;**15**:70−7.
244. Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, Jin S, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes Dev* 2007;**21**:1621−35.
245. Elgendy M, Minucci S. A novel autophagy-independent, oncosuppressive function of BECN1: degradation of MCL1. *Autophagy* 2015;**11**:581−2.
246. Cicchini M, Chakrabarti R, Kongara S, Price S, Nahar R, Lozy F, et al. Autophagy regulator BECN1 suppresses mammary tumorigenesis driven by WNT1 activation and following parity. *Autophagy* 2014;**10**:2036−52.
247. Rohatgi RA, Shaw LM. An autophagy-independent function for Beclin 1 in cancer. *Mol Cell Oncol* 2016;**3**:e1030539.
248. Rohatgi RA, Janusis J, Leonard D, Bellvé KD, Fogarty KE, Baehrecke EH, et al. Beclin 1 regulates growth factor receptor signaling in breast cancer. *Oncogene* 2015;**34**:5352−62.
249. Matthew-Onabanjo AN, Janusis J, Mercado-Matos J, Carlisle AE, Kim D, Levine F, et al. Beclin 1 promotes endosome recruitment of hepatocyte growth factor tyrosine kinase substrate to suppress tumor proliferation. *Cancer Res* 2020;**80**:249−62.
250. Wang W, Fan H, Li X, Wu G, Zhao W, Zhang G, et al. Beclin 1 promotes apoptosis and decreases invasion by upregulating the expression of ECRG4 in A549 human lung adenocarcinoma cells. *Mol Med Rep* 2016;**14**:355−60.
251. Kang R, Zhu S, Zeh HJ, Klionsky DJ, Tang D. BECN1 is a new driver of ferroptosis. *Autophagy* 2018;**14**:2173−5.
252. Wu CL, Zhang SM, Lin L, Gao SS, Fu KF, Liu XD, et al. BECN1-knockout impairs tumor growth, migration and invasion by suppressing the cell cycle and partially suppressing the epithelial−mesenchymal transition of human triple-negative breast cancer cells. *Int J Oncol* 2018;**53**:1301−12.
253. Shen H, Yin L, Deng G, Guo C, Han Y, Li Y, et al. Knockdown of Beclin-1 impairs epithelial−mesenchymal transition of colon cancer cells. *J Cell Biochem* 2018;**119**:7022−31.
254. Ye C, Yu X, Liu X, Zhan P, Nie T, Guo R, et al. Beclin-1 knockdown decreases proliferation, invasion and migration of Ewing sarcoma SK-ES-1 cells *via* inhibition of MMP-9. *Oncol Lett* 2018;**15**:3221−5.
255. Cheng Z, Xin H, Han T. BECN1 promotes the migration of NSCLC cells through regulating the ubiquitination of vimentin. *Cell Adhes Migrat* 2019;**13**:249−59.
256. Duan S. Silencing the autophagy-specific gene Beclin-1 contributes to attenuated hypoxia-induced vasculogenic mimicry formation in glioma. *Cancer Biomarkers* 2018;**21**:565−74.
257. Chen L, Liu D, Zhang Y, Zhang H, Cheng H. The autophagy molecule Beclin 1 maintains persistent activity of NF-κB and Stat3 in HTLV-1-transformed T lymphocytes. *Biochem Biophys Res Commun* 2015;**465**:739−45.
258. Frémont S, Gérard A, Galloux M, Janvier K, Karess RE, Berlioz-Torrent C. Beclin-1 is required for chromosome congression and proper outer kinetochore assembly. *EMBO Rep* 2013;**14**:364−72.
259. Pan Y, Zhao Z, Li J, Li J, Luo Y, Li W, et al. Nuclear Beclin 1 destabilizes retinoblastoma protein to promote cell cycle progression and colorectal cancer growth. *Cancers* 2022;**14**:4735.
260. Boo SJ, Piao MJ, Kang KA, Zhen AX, Fernando P, Herath H, et al. Comparative study of autophagy in oxaliplatin-sensitive and resistant SNU-C5 colon cancer cells. *Biomol Ther (Seoul)* 2022;**30**:447−54.
261. Zhang J, Mao W, Liu Y, Ding J, Wang J, Yu Z, et al. 3-MA enhanced chemosensitivity in cisplatin resistant hypopharyngeal squamous carcinoma cells *via* inhibiting Beclin -1 mediated autophagy. *Curr Pharmaceut Des* 2021;**27**:996−1005.
262. Zhang M, Sun Y, Meng J, Zhang L, Liang C, Chang C. Targeting AR-Beclin 1 complex-modulated growth factor signaling increases the antiandrogen Enzalutamide sensitivity to better suppress the castration-resistant prostate cancer growth. *Cancer Lett* 2019;**442**:483−90.
263. Yang X, Bai F, Xu Y, Chen Y, Chen L. Intensified Beclin-1 mediated by low expression of Mir-30a-5p promotes chemoresistance in human small cell lung cancer. *Cell Physiol Biochem* 2017;**43**:1126−39.
264. Dai S, Yang S, Hu X, Sun W, Tawa G, Zhu W, et al. 17-Hydroxy Wortmannin restores TRAIL's response by ameliorating increased Beclin 1 level and autophagy function in TRAIL-resistant colon cancer cells. *Mol Cancer Therapeut* 2019;**18**:1265−77.
265. Chen W, Li Z, Liu H, Jiang S, Wang G, Sun L, et al. MicroRNA-30a targets BECLIN-1 to inactivate autophagy and sensitizes gastrointestinal stromal tumor cells to imatinib. *Cell Death Dis* 2020;**11**:198.
266. Xia J, He Y, Meng B, Chen S, Zhang J, Wu X, et al. NEK2 induces autophagy-mediated bortezomib resistance by stabilizing Beclin-1 in multiple myeloma. *Mol Oncol* 2020;**14**:763−78.
267. Babaei G, Aziz SG, Jaghi NZZ. EMT, cancer stem cells and autophagy; the three main axes of metastasis. *Biomed Pharmacother* 2021;**133**:110909.
268. Duan H, Liu Y, Gao Z, Huang W. Recent advances in drug delivery systems for targeting cancer stem cells. *Acta Pharm Sin B* 2021;**11**:55−70.
269. Gong C, Song E, Codogno P, Mehrpour M. The roles of BECN1 and autophagy in cancer are context dependent. *Autophagy* 2012;**8**:1853−5.
270. Bie Q, Song H, Chen X, Yang X, Shi S, Zhang L, et al. IL-17B/IL-17RB signaling cascade contributes to self-renewal and tumorigenesis of cancer stem cells by regulating Beclin-1 ubiquitination. *Oncogene* 2021;**40**:2200−16.
271. Zhu Y, Huang S, Chen S, Chen J, Wang Z, Wang Y, et al. SOX2 promotes chemoresistance, cancer stem cells properties, and

epithelial-mesenchymal transition by β-catenin and Beclin1/autophagy signaling in colorectal cancer. *Cell Death Dis* 2021;**12**:449.
272. Zhuang W, Long L, Zheng B, Ji W, Yang N, Zhang Q, et al. Curcumin promotes differentiation of glioma-initiating cells by inducing autophagy. *Cancer Sci* 2012;**103**:684−90.
273. Tao Z, Li T, Ma H, Yang Y, Zhang C, Hai L, et al. Autophagy suppresses self-renewal ability and tumorigenicity of glioma-initiating cells and promotes Notch1 degradation. *Cell Death Dis* 2018;**9**:1063.
274. Brunel A, Hombourger S, Barthout E, Battu S, Kögel D, Antonietti P, et al. Autophagy inhibition reinforces stemness together with exit from dormancy of polydisperse glioblastoma stem cells. *Aging (Albany N Y)* 2021;**13**:18106−30.
275. Kasprzak A. The role of tumor microenvironment cells in colorectal cancer (CRC) cachexia. *Int J Mol Sci* 2021;**22**:1565.
276. Swartz MA, Iida N, Roberts EW, Sangaletti S, Wong MH, Yull FE, et al. Tumor microenvironment complexity: emerging roles in cancer therapy. *Cancer Res* 2012;**72**:2473−80.
277. Mowers EE, Sharifi MN, Macleod KF. Functions of autophagy in the tumor microenvironment and cancer metastasis. *FEBS J* 2018;**285**:1751−66.
278. Song J, Guo X, Xie X, Zhao X, Li D, Deng W, et al. Autophagy in hypoxia protects cancer cells against apoptosis induced by nutrient deprivation through a Beclin1-dependent way in hepatocellular carcinoma. *J Cell Biochem* 2011;**112**:3406−20.
279. Sanchez CG, Penfornis P, Oskowitz AZ, Boonjindasup AG, Cai DZ, Dhule SS, et al. Activation of autophagy in mesenchymal stem cells provides tumor stromal support. *Carcinogenesis* 2011;**32**:964−72.
280. Morikawa A, Takeuchi T, Kito Y, Saigo C, Sakuratani T, Futamura M, et al. Expression of beclin-1 in the microenvironment of invasive ductal carcinoma of the breast: correlation with prognosis and the cancer-stromal interaction. *PLoS One* 2015;**10**:e0125762.
281. Wu SY, Fu T, Jiang YZ, Shao ZM. Natural killer cells in cancer biology and therapy. *Mol Cancer* 2020;**19**:120.
282. Baginska J, Viry E, Berchem G, Poli A, Noman MZ, van Moer K, et al. Granzyme B degradation by autophagy decreases tumor cell susceptibility to natural killer-mediated lysis under hypoxia. *Proc Natl Acad Sci U S A* 2013;**110**:17450−5.
283. Noman MZ, Berchem G, Janji B. Targeting autophagy blocks melanoma growth by bringing natural killer cells to the tumor battlefield. *Autophagy* 2018;**14**:730−2.
284. Boutilier AJ, Elsawa SF. Macrophage polarization states in the tumor microenvironment. *Int J Mol Sci* 2021;**22**:6995.
285. Wen ZF, Liu H, Gao R, Zhou M, Ma J, Zhang Y, et al. Tumor cell-released autophagosomes (TRAPs) promote immunosuppression through induction of M2-like macrophages with increased expression of PD-L1. *J Immunother Cancer* 2018;**6**:151.
286. Shan M, Qin J, Jin F, Han X, Guan H, Li X, et al. Autophagy suppresses isoprenaline-induced M2 macrophage polarization *via* the ROS/ERK and mTOR signaling pathway. *Free Radic Biol Med* 2017;**110**:432−43.
287. Sutton MN, Huang GY, Liang X, Sharma R, Reger AS, Mao W, et al. DIRAS3-derived peptide inhibits autophagy in ovarian cancer cells by binding to Beclin1. *Cancers* 2019;**11**:557.
288. Wang Y, Tai X, Zhang L, Liu Y, Gao H, Chen J, et al. A novel antitumour strategy using bidirectional autophagic vesicles accumulation *via* initiative induction and the terminal restraint of autophagic flux. *J Control Release* 2015;**199**:17−28.
289. Wu S, He Y, Qiu X, Yang W, Liu W, Li X, et al. Targeting the potent Beclin 1-UVRAG coiled-coil interaction with designed peptides enhances autophagy and endolysosomal trafficking. *Proc Natl Acad Sci U S A* 2018;**115**:E5669−78.
290. Chen J, Zhang X, Gao S, Li N, Keng V, Zhao Y. A Beclin 1-targeting stapled peptide synergizes with erlotinib to potently inhibit proliferation of non-small-cell lung cancer cells. *Biochem Biophys Res Commun* 2022;**636**:125−31.
291. Yang Q, Qiu X, Zhang X, Yu Y, Li N, Wei X, et al. Optimization of Beclin 1-targeting stapled peptides by staple scanning leads to enhanced antiproliferative potency in cancer cells. *J Med Chem* 2021;**64**:13475−86.
292. Zhao R, Jin W, Jiang X, Yuan Z, Liu B, Fu L. Discovery of a small-molecule activator of beclin-1 that induces autophagy-associated cell death and apoptosis in triple negative breast cancer. *Acta Pharm Sin B* 2021;**56**:1369−83.
293. Huangfu L, Wang X, Tian S, Chen J, Wang X, Fan B, et al. Piceatannol enhances Beclin-1 activity to suppress tumor progression and its combination therapy strategy with everolimus in gastric cancer. *Sci China Life Sci* 2022;**66**:298−312.
294. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. *Nature* 2021;**596**:583−9.
295. Ding GB, Sun J, Wu G, Li B, Yang P, Li Z, et al. Robust anticancer efficacy of a biologically synthesized tumor acidity-responsive and autophagy-inducing functional Beclin 1. *ACS Appl Mater Interfaces* 2018;**10**:5227−39.
296. Kochneva G, Zonov E, Grazhdantseva A, Yunusova A, Sibolobova G, Popov E, et al. Apoptin enhances the oncolytic properties of vaccinia virus and modifies mechanisms of tumor regression. *Oncotarget* 2014;**5**:11269−82.
297. Lv C, Su Q, Liang Y, Hu J, Yuan S. Oncolytic vaccine virus harbouring the *IL-24* gene suppresses the growth of lung cancer by inducing apoptosis. *Biochem Biophys Res Commun* 2016;**476**:21−8.
298. Zhou S, Zhang M, Zhang J, Shen H, Tangsakar E, Wang J. Mechanisms of Apoptin-induced cell death. *Med Oncol* 2012;**29**:2985−91.
299. Lei W, Wang S, Xu N, Chen Y, Wu G, Zhang A, et al. Enhancing therapeutic efficacy of oncolytic vaccinia virus armed with Beclin-1, an autophagic gene in leukemia and myeloma. *Biomed Pharmacother* 2020;**125**:110030.
300. Li X, Song Y. Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. *J Hematol Oncol* 2020;**13**:50.
301. Winkle M, El-Daly SM, Fabbri M, Calin GA. Noncoding RNA therapeutics—challenges and potential solutions. *Nat Rev Drug Discov* 2021;**20**:629−51.
302. Lu B, Ye J. Commentary: PROTACs make undruggable targets druggable: challenge and opportunity. *Acta Pharm Sin B* 2021;**11**:3335−6.
303. An Z, Chiang WC, Fernández Á F, Franco LH, He C, Huang SY, et al. Beth Levine's Legacy: from the discovery of BECN1 to therapies. A Mentees' perspective. *Front Cell Dev Biol* 2022;**10**:891332.
304. Calis S, Dogan B, Durdagi S, Celebi A, Yapicier O, Kilic T, et al. A novel BH3 mimetic Bcl-2 inhibitor promotes autophagic cell death and reduces *in vivo* glioblastoma tumor growth. *Cell Death Dis* 2022;**8**:433.
305. Pavlinov I, Salkovski M, Aldrich LN. Beclin 1-ATG14L protein−protein interaction inhibitor selectively inhibits autophagy through disruption of VPS34 complex I. *J Am Chem Soc* 2020;**142**:8174−82.
306. Wu D, Li Y, Zheng L, Xiao H, Ouyang L, Wang G, et al. Small molecules targeting protein−protein interactions for cancer therapy. *Acta Pharm Sin B* 2023;**13**:4060−88.