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Regulation of Autophagy by Beclin 1 in the Heart

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Abstract

Dysregulation of autophagy in cardiomyocytes is implicated in various heart disease conditions. Beclin 1, a mammalian ortholog of yeast Atg6 and a core component of the autophagy machinery, plays a central role in the regulation of autophagy through activation of Vps34. Beclin 1's ability to activate Vps34 is tightly regulated via transcriptional regulation, miRNA, post-translational modification, and interaction with Beclin 1 binding proteins. Of these mechanisms, binding of Beclin 1 with Bcl-2 family proteins (Bcl-2/X_L) that negatively regulate autophagy activity has been shown to be both positively and negatively regulated by various kinases, including DAPK, ROCK1, Mst1 and JNK1, in response to external stimuli. Beclin 1's interaction with Bcl-2/X_L also secondarily affects apoptosis through regulation of pro-apoptotic BH3 domain containing proteins. Thus, modulation of Beclin 1 significantly influences both autophagy and apoptosis, thereby deeply affecting the survival and death of cardiomyocytes in the heart. In this review, we discuss the signaling mechanism of autophagy modulation through Beclin 1 and therapeutic potential of Beclin 1 in heart diseases.

Keywords

Autophagy; Beclin 1; Bcl-2 family proteins; Phosphorylation; Apoptosis

The role of autophagy in the heart

Accumulating lines of evidence suggest that vigorous protein quality control (PQC) systems are essential for maintaining the long-term wellbeing of non-proliferating mammalian cells, such as neurons and cardiomyocytes (CMs). Autophagy, a catabolic mechanism that degrades long-lived cytosolic proteins and organelles, is one of the major components of the PQC systems [1]. Heart-specific ablation of Atg5, a major regulatory molecule of autophagy,

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Disclosures

None.

in mice caused impairment of cardiac function with increased deposition of protein aggregates at baseline and during stress, suggesting that autophagy plays a salutary role in the heart [2]. Autophagy is observed in the heart at baseline and in response to stress, such as ischemia, and during heart failure. However, accumulation of protein aggregates and dysfunctional organelles is commonly observed in a variety of heart diseases, including hemodynamic stress-induced cardiac hypertrophy, chronic myocardial infarction (MI), and dilated cardiomyopathy (DCM) [3, 4], suggesting that activation of autophagy may not be sufficient in these conditions. In fact, a recent investigation revealed that enhancing autophagy ameliorates desmin-related cardiomyopathies (DRCs), inherited cardiomyopathies that result in severe heart failure due to protein aggregation and myofibrillar disarray in CMs [5]. Thus, autophagy plays an important role in protecting normal cardiac function by maintaining optimal PQC.

Although activation of autophagy is generally protective in the heart in many conditions, it can also induce cell death and cardiac dysfunction in a context-dependent manner. For example, ischemia/reperfusion (I/R) upregulates autophagy in the mouse heart [6]. In this case, suppression of autophagy attenuates myocardial injury during I/R [6], suggesting that upregulation of autophagy during I/R could be detrimental. Similarly, pressure overload (PO) activates autophagy at least at an acute phase and suppression of PO-induced autophagy alleviates pathological cardiac remodeling [7]. Although how activation of autophagy can cause detrimental effects in the heart remains to be elucidated, activation of autophagy can cause a unique form of cell death termed autosis in murine bone marrow-derived macrophages, primary MEF cells and HeLa cells in response to starvation in vitro and hippocampal neurons of neonatal rats subjected to cerebral hypoxia–ischemia in vivo [12]. It should be noted, however, that conflicting results have been reported regarding whether autophagosome formation and autophagic flux are stimulated in response to I/R and PO [8–11]. Furthermore, it has not yet been firmly established that autophagy has direct detrimental effects upon the heart during reperfusion and PO, whereas it is clearly protective under other conditions. Thus, further investigation is needed to clarify the role of autophagy during I/R and PO.

Regardless of the controversy in some areas, it is fair to state that autophagy has a significant influence upon the function and the survival of cardiomyocytes in the heart (Figure 1). It is therefore important to accurately evaluate whether autophagy is stimulated or inhibited in a given condition, to understand the signaling mechanism through which autophagy is regulated in cardiomyocytes under various conditions and to elucidate its functional significance [13, 14]. Here we discuss the role of Beclin 1, a key molecule in the autophagic machinery, in regulating the level of autophagy and the survival and death of cardiomyocytes and the underlying mechanisms.

Beclin 1: a critical autophagy regulator in the heart

Mammalian Beclin 1, an ortholog of the Atg6/vacuolar protein sorting (Vps)-30 protein in yeast, plays an important role in a critical step of the autophagic process, namely, autophagosome formation, by interacting with the class III-type phosphoinositide 3-kinase (Class III PI3K, also known as Vps34). Activation of the kinase activity of the Beclin 1-

Vps34 complex promotes production of phosphatidylinositol 3-phosphate, thereby facilitating lipid membrane extension, cargo recruitment and autophagosome maturation [15]. Beclin 1 and Vps34 form two distinct complexes. Beclin 1-Vps34 complex I, in which Atg14L cross-links between Beclin 1 and the Vps34-p150 complex, mediates autophagosome formation. On the other hand, Beclin 1-Vps34 complex II, in which Beclin 1 and the Vps34-p150 complex are bridged by UVRAG, regulates the vacuolar protein-sorting pathway (Figure 2) [16]. The Beclin 1-Vps34 complex I is one of the most important platforms for regulating autophagy activity by various cellular stimuli [17]. The kinase activity of the Beclin 1-Vps34 complex I is regulated via post-translational modification or direct binding with other proteins. These modifications lead to sequestration of Beclin 1 from specific subcellular locations, such as Golgi apparatus or cytoskeleton, or alteration of its interactions with the binding partners of the Beclin 1-Vps34 complex I, including Atg14 and Bcl-2 family proteins [18]. On the other hand, Beclin 1 negatively regulates autophagosome-lysosome fusion in co-operation with Rubicon, which is blocked by virus-derived proteins, including influenza M2 and the HIV-1 Nef proteins[19–21].

Changes in the protein level of Beclin 1 in the heart are observed in many disease conditions. In the swine model of hibernating myocardium subjected to repetitive myocardial stunning, the amount of Beclin 1 in the heart was markedly increased [22]. Although autophagy plays a protective role in the heart in this condition, the causative involvement of Beclin 1 upregulation in the development of autophagy and cardioprotection in hibernating myocardium remains to be shown [22–24]. On the other hand, reperfusion induces massive autophagy that causes detrimental effects in the hearts through reactive oxygen species (ROS)-mediated strong upregulation of Beclin 1 [6, 25–27]. One mechanism by which ROS transcriptionally upregulate Beclin 1 is stimulation of NF-κB [28, 29]. Activation of autophagy in response to PO also exacerbates pathological cardiac hypertrophy [7]. Some endogenous factors, including microRNAs and transcriptional factors, modulate autophagy during PO by regulating Beclin 1 levels [30]. Hypertrophic stimuli, such as PO and angiotensin II, downregulate miR-30a, which stimulates autophagy through upregulation of Beclin 1 and exacerbates cardiac hypertrophy [31, 32]. Activating transcription factor 3 (ATF3), a member of the cAMP response element-binding protein/ATF family of transcription factors, protects against PO-induced heart failure through suppression of Beclin 1 expression [33]. ATF3 binds to the ATF/cAMP response element of the Beclin 1 promoter, thereby suppressing autophagy through downregulation of the Beclin 1-dependent pathway. Taken together, these results suggest that changes in Beclin 1 levels significantly affect the function and survival of cardiomyocytes, which either positively or negatively correlates with the level of autophagy. However, whether these effects of Beclin 1 are mediated primarily through changes in autophagy activity remains to be shown [30].

Regulation of Beclin 1 by post-translational modifications

A growing body of evidence suggests that binding of Bcl-2 family proteins, such as Bcl-2 and Bcl-X_L (Bcl-2/X_L), to Beclin 1, which was originally cloned as a novel Bcl-2-homology (BH)-3 domain-only protein [34], negatively regulates autophagosome formation by promoting the dissociation between Beclin 1 and Vps34 [13]. Each Beclin 1 molecule interacts with two Bcl-2/X_L molecules: The BH3 domain of Beclin 1 binds to the

hydrophobic groove (the BH3 binding domain) of one Bcl-2/X_L molecule whereas the coiled-coil domain (CCD) of Beclin 1 binds to the BH4 domain of the other Bcl-2/X_L molecule [4, 35].

The CCD of Beclin 1 acts as a central interaction platform for either formation of the inactive homodimer or heterodimerization with Atg14L or UVRAG to stimulate autophagy or vacuolar protein sorting. Crystallographic analyses showed that the CCDs of two Beclin 1 molecules can associate with one another in antiparallel orientation [36]. Importantly, among 13 leucine-rich heptad-repeat motifs in the CCD of Beclin 1, only 7 pairs have ideal hydrophobic interfaces that can stabilize the dimer, whereas the remaining 6 pairs have "imperfect" interfaces in the Beclin 1 homodimer. In addition, the Beclin 1 CCD has a series of repulsive electrostatic interactions between heptad repeats, which weaken dimerization stability [37]. The high proportion of negatively charged amino acids in the Beclin 1 CCD causes a strong destabilizing effect on the Beclin 1 homodimer. Thus, Beclin 1 homodimer is metastable and readily transits into an active heterodimer complex with other CCD-containing proteins, such as Atg14L or UVRAG, which, in turn, promote autophagy or vacuolar protein sorting.

Unlike the BH1-3 domains, BH4 is conserved only among the anti-apoptotic Bcl-2 family proteins, namely Bcl-2/X_L, and can bind with non-Bcl-2 family proteins, including voltage-dependent anion channel and inositol 1,4,5-trisphosphate receptor, thereby modulating the functions of its binding partners [38, 39]. The α-helical structure of the BH4 domain, which has well-conserved hydrophobic amino acids, might be important for interaction with other proteins [38]. Furthermore, there are also well-conserved positively charged amino acids in the BH4 domain of Bcl-2 family proteins, which may neutralize the repulsive electrostatic interactions of the heptad repeats in the Beclin 1 homodimer. Thus, in theory, two Bcl-2 molecules form bridges between two Beclin 1 molecules aligned with their CCDs in an anti-parallel orientation. When the interaction between the BH3 domain of Beclin 1 and the BH3 binding domain of Bcl-2/X_L is stimulated, Bcl-2 acts to enhance homodimerization of Beclin 1 by stabilizing dimerization of their CCD in an anti-parallel orientation. These results suggest that the strength of the interaction between the BH3 domain of Beclin 1 and the BH3 binding domain of Bcl-2/X_L determines whether Beclin 1 homodimerizes, thereby taking an inactive confirmation, or heterodimerizes with Atg14L, thereby taking an active conformation.

Increasing lines of evidence suggest that the interaction between Beclin 1 and Bcl-2/X_L is regulated by post-translational modifications, such as phosphorylation and ubiquitination [40–43]. The binding interface of Beclin 1 and Bcl-2/X_L, specifically, the BH3 domain of Beclin 1 and the hydrophobic groove of Bcl-2/X_L, could be a hot spot of such modifications. Mammalian sterile 20-like kinase 1 (Mst1), a pro-apoptotic serine/threonine kinase, phosphorylates Beclin 1 in its BH3 domain at Thr^{108}, promoting binding of Beclin 1 with Bcl-2/X_L [4]. Phosphorylation of Beclin 1-Thr^{108}, which confers a negative charge to this residue, enhances its interaction with a histidine residue of Bcl-2/X_L, a positively-charged basic amino acid that is located on the edge of its hydrophobic groove [44], thereby promoting interaction between Beclin 1 and Bcl-2/X_L. Mst1 is activated in response to cellular stress and promotes the progression of cardiac dysfunction by stimulating apoptosis

[45–47]. Inhibition of endogenous Mst1 dramatically reduces the accumulation of aggresomes and cardiac dysfunction by stimulating autophagy in a chronic MI model. Furthermore, activation of Mst1, Thr^{108} phosphorylation of Beclin 1, attenuation of autophagy, and accumulation of protein aggregates are all increased in human end-stage DCM hearts.

In contrast, phosphorylation of Thr^{119} in the BH3 domain of Beclin 1 by death-associated protein kinase (DAPK) or Rho-associated coiled-coil containing protein kinase 1 (ROCK1) inhibits binding of Beclin 1 with Bcl-2/X_L [41]. Multisite phosphorylation of Bcl-2 in its nonstructural loop (Thr^{69}, Ser^{70}, Ser^{87}) by c-Jun N-terminal kinase 1 (JNK1) also promotes dissociation of Beclin 1 from Bcl-2 that results in induction of autophagy [40, 48]. Phosphorylation of Bcl-2 in response to starvation is mediated by JNK1 [40]. In addition, exercise also promotes phosphorylation of Bcl-2 by undetermined kinase(s) [48]. In homozygous knock-in mice in which Thr^{69}, Ser^{70} and Ser^{87} are replaced with alanine in combination (Bcl-2^{AAA} mice), multiple phosphorylation sites of Bcl-2 are not phosphorylated by upstream kinases even in the presence of starvation or exercise stimuli. In these mice, exercise-induced autophagy is suppressed, indicating the importance of phosphorylation of Bcl-2 at these sites for induction of autophagy by exercise [48]. The enhancement of insulin sensitivity in skeletal muscles in response to acute exercise was also significantly attenuated in Bcl-2^{AAA} mice compared to in wild type mice. In addition, the beneficial effects of chronic exercise against high-fat diet-induced diabetes observed in wild type mice were abolished in Bcl-2^{AAA} mice, indicating the involvement of Beclin 1-mediated autophagy in regulation of insulin signaling mechanisms.

The interaction between Bcl-2/X_L and Beclin 1 that stabilizes the Beclin 1 homodimer also suppresses phosphorylation of Beclin 1 at Ser^{90} by MAPKAPK2 and MAPKAPK3 (MK2/MK3), thereby inhibiting autophagy [49]. The formation of Beclin 1-Atg14L heterodimer facilitates pro-autophagic phosphorylation, including phosphorylation of Beclin 1 by unc-51 like autophagy activating kinase 1 (ULK1) and AMP-activated protein kinase (AMPK) (Figure 3). Under nutrient-rich conditions, the mTORC1 complex phosphorylates ULK1 at Ser^{757}, thereby inhibiting ULK1 activity [50]. Conversely, AMPK activated by low-glucose conditions upregulates autophagy by inhibiting mTORC1 activity and by directly activating ULK1 through phosphorylation at Ser^{317}, Ser^{555} and Ser^{777} [50, 51]. Consequently, ULK1 phosphorylates Beclin 1 at Ser^{14}, thereby promoting Beclin 1-Atg14L heterodimer formation [52]. Activated AMPK also directly enhances formation of Beclin 1-Vps34 complex I by phosphorylating Beclin 1 at Ser^{93} and Ser^{96} [53].

Akt and epidermal growth factor receptor (EGFR) phosphorylate Beclin 1 in its CCD or beta-alpha repeated autophagy-specific (BARA) domain. Akt-mediated phosphorylation of Beclin 1 at Ser^{234} and Ser^{295} promotes the formation of the Beclin 1/14-3-3/vimentin intermediate filament complex, thereby inhibiting autophagy [54]. Activated EGFR phosphorylates Beclin 1 at Tyr^{229}, Tyr^{233} and Tyr^{352}, which promotes Beclin 1 homodimerization and Rubicon binding and suppresses formation of the autophagy-active Beclin 1-Vps34 complex I [55]. As two of the three sites at which Beclin 1 is phosphorylated by EGFR, Tyr^{229} and Tyr^{233}, are located at the interface of the Beclin 1 CCD, phosphorylation of these two tyrosine residues stabilizes the metastable antiparallel

homodimer by altering the electrostatic power-dependent molecular bonding [36, 56]. Unlike active EGFR, inactive EGFR promotes basal and starvation-induced autophagy activity [57]. Inactive EGFR forms a complex with lysosomal protein transmembrane 4b (LAPTM4B) and exocyst complex component 2 (EXOC2) that localizes in endosomes and enhances interaction of EGFR with Rubicon. The formation of the EGFR/LAPTM4B/EXOC2 complex leads to Beclin 1 dissociation from Rubicon, thereby initiating autophagy. Either Akt or EGFR-mediated phosphorylation of Beclin 1 is known to contribute to oncogenic transformation in tumor cells by suppressing the Beclin 1-mediated autophagy machinery. However, the role of the inhibitory effects of Akt or EGFR on autophagy in the heart remains to be elucidated.

Cross-talk between apoptosis and autophagy via Beclin 1-mediated signaling

The levels of autophagy and apoptosis inversely correlate with one another in the heart in the presence of chronic hypoxia [22]. Although activation of autophagy presumably inhibits apoptosis due to a general improvement of cellular conditions, autophagy and apoptosis also directly affect one another by modulating autophagy/apoptosis regulatory mechanisms.

Unlike other BH3-only Bcl-2 family proteins, Beclin 1 does not itself possess any direct pro-apoptotic function. However, Beclin 1 plays an important role as a “crossroad of cell death” by modulating both autophagy and apoptosis. Beclin 1 acts as an anti-apoptotic protein under certain conditions, such as nutrient deprivation, irradiation and hypoxia. Depletion of *bec-1*, a homolog of Beclin 1, triggers CED-3/caspase-dependent programmed cell death in *Caenorhabditis elegans* [58]. In addition, knockdown of Bid, a pro-apoptotic Bcl-2 family protein, upregulates Beclin 1 expression, thereby suppressing cell death in breast cancer MCF-7 cells by inhibiting apoptosis and inducing autophagy [59]. However, Beclin 1 can also act in some cases as an enhancer of pro-apoptotic signaling. Mst1 is known as a potent protein kinase that promotes apoptosis in CMs via several signaling pathways [45–47]. We recently found a novel Mst1-mediated pro-apoptotic mechanism [4] in which phosphorylation of Beclin 1 by Mst1 at Thr^{108} markedly enhances the binding affinity of Bcl-2/X_L to Beclin 1, resulting in displacement of Bcl-2 from Bax and increases in the amount of active Bax, which, in turn, enhances the apoptotic machinery.

Conversely, pro-apoptotic signaling sometimes negatively regulates the autophagy machinery. Bax-induced pro-apoptotic machinery suppresses autophagy by facilitating cleavage of Beclin 1 at Asp^{149} by caspases [60]. Bim (Bcl-2 interacting mediator of cell death) mediates sequestration of Beclin 1 from microtubules, thereby interfering with the autophagy machinery [61]. Moreover, tumor necrosis factor related apoptosis-inducing ligand (TRAIL) also triggers the caspase-mediated cleavage of Beclin 1 in HeLa cells [62].

Increasing lines of evidence suggest that a negative feedback mechanism may cross-regulate autophagy and apoptosis. Caspase-8 activated by death receptor can be degraded by autophagy [63], and truncated DeltaN63 Atg4D, a cleavage product of Atg4D by caspase-3, facilitates autophagic activity by stimulating the delipidation of gamma-aminobutyric acid receptor-associated protein-like 1 (GABARAP-L1) [64]. Based on these lines of evidence,

we hypothesize that Beclin 1 and its partners that can modulate both autophagy and apoptosis are promising therapeutic targets for regulating cell life or death (Figure 4).

Targeting Beclin 1-mediated autophagy regulation as a therapeutic strategy for heart diseases

As the alteration of autophagy activity plays a critical role in the progression of various pathological conditions, modulation of autophagy activity represents a potential therapeutic target for human diseases. Currently, a number of clinical trials targeting autophagy have been carried out to treat cancers [65], amyotrophic lateral sclerosis [66] and α1-antitrypsin deficiency liver cirrhosis [67]. There are currently no active clinical trials aimed at testing whether pro- or anti-autophagic interventions are effective in heart diseases. However, several promising therapeutic modulators against autophagy activity have been identified.

An increasing number of experimental Beclin 1 gene therapy studies suggest that modulation of Beclin 1 activity could be therapeutically beneficial in a cystic fibrosis model [68], collagen VI muscular dystrophy models [69], an alpha-synuclein model of Parkinson's disease [70] and a spinocerebellar ataxia type 3 disease model [71]. Similarly, the pathological condition of heart disease models were alleviated by several existing drugs, such as chloramphenicol and urocortin, by regulating Beclin 1 expression [25, 72]. The mechanism that modulates the Beclin 1-Bcl-2/X_L interaction represents one of the most likely molecular targets for regulating the Beclin 1-mediated autophagy machinery. BH3 mimetic agents, such as ABT737, disrupt Beclin 1-Bcl-2/X_L interactions by pharmacological antagonism [73, 74]. However, as BH3 mimetics would broadly antagonize the BH3 domain of any BH3-containing proteins, it is possible that such agents could affect not only Beclin 1-mediated autophagy but also apoptotic activity that are mediated through other BH3-containing proteins. Therefore, more selective BH3 modulators are needed that can specifically inhibit the anti-autophagy activity of Bcl-2/X_L without influencing the pro-apoptotic signaling regulated by BH3-containing proteins. In this regard, a small molecule that can inhibit Beclin 1-Bcl-2/X_L interactions by blocking a phosphorylation site, such as Beclin 1-Thr^{108} [4], would be a promising compound for inducing autophagy. Such a compound could be used to treat conditions in which selective autophagy activation is beneficial, such as neurodegenerative diseases and proteotoxicity-associated heart diseases.

A novel autophagy inducer, Tat-Beclin 1, was recently produced [75]. Tat-Beclin 1 is a cell permeable peptide derived from the BARA domain of Beclin 1 (267–284) that binds with human immunodeficiency virus-1 Nef. Golgi-associated plant pathogenesis-related protein 1 (GAPR-1) is a cellular protein that associates with lipid rafts at the membrane of the Golgi complex. Under basal condition, GAPR-1 anchors Beclin 1 firmly to the Golgi complex to inhibit the autophagic properties of Beclin 1. Interestingly, Tat-Beclin 1 competitively inhibits the binding of Beclin 1 to GAPR-1 on the Golgi membranes. Consequently, Beclin 1 is released from the Golgi into the cytoplasm, allowing it to mediate the formation of autophagosomes [75]. Tat-Beclin 1 suppresses the accumulation of htt103Q, a polyglutamine expansion protein derived from human mutant Huntingtin protein, and the replication of several pathogens, including HIV-1 and West Nile virus, both *in vitro* and *in*

vivo. As the efficacy of Tat-Beclin 1 for inducing autophagy is strong, it may be useful for the treatment of heart diseases caused by insufficient autophagy activity. However, excessive induction of autophagy by treatment with Tat-Beclin 1 results in cell death [12]. This type of cell death, termed “autosis”, is distinguished from either apoptosis or necroptosis by its unique morphological characteristics. When neonatal rats were subjected to cerebral ischemia, autosis emerged in hippocampal neurons. Screening of a compound library identified cardiac glycosides, antagonists of Na^+/K^+-ATPase, as effective suppressors of autosis [12]. Although the mechanisms by which cardiac glycosides inhibit autosis remain to be elucidated, cardiac glycosides effectively attenuated autotic cell death *in vivo*. If autosis plays a critical role in the heart under conditions in which excessive autophagy has been shown to be detrimental, such as I/R [6, 26] and acute PO [7], cardiac glycosides could be promising therapeutic agents. Thus, given that recent advances in elucidation of the detailed molecular function of Beclin 1 and progress in the methods of drug discovery are dramatic, it is possible that more specific Beclin 1 modulators will emerge [76].

In addition to pharmacological intervention, recent progress in autophagy research suggests that non-pharmacological autophagy regulation also has potential applications for controlling various diseases. Physical exercise is known to have beneficial effects on established risk factors for cardiovascular diseases, such as diabetes and dyslipidemia. The discovery that exercise induces autophagy through the phosphorylation of Bcl-2 [48] added a novel favorable effect of exercise against heart diseases. In fact, voluntary exercise improves survival of a desmin-related cardiomyopathy mouse model in the presence of Atg7 by stimulating autophagy, suggesting that exercise-induced stimulation of autophagy may be a viable therapeutic strategy for improving cardiac performance under proteotoxic conditions [5].

Concluding remarks

Since Beclin 1 is a key molecule in the control of autophagic activity, its activity is regulated by multiple mechanisms, including post-translational modification, protein-protein interaction and subcellular localization. During the past few years, we have witnessed dramatic advancement in the understanding of the structure and function of the Beclin 1-Vps34 complex. The interaction between Beclin 1 and Bcl-2/X_L has been particularly well characterized and represents a promising molecular target of medical therapy to modulate autophagy. Importantly, alteration of Beclin 1 activity through modulation of its interaction with Bcl-2/X_L potentially affects not only autophagy but also apoptosis by indirectly modulating interaction between Bcl-2/X_L and Bax. Therefore, more studies regarding the structure and function of Beclin 1 in conjunction with its connection with the apoptotic machinery appear essential in order to apply what is known for the effective treatment of heart disease through modulation of autophagy by targeting Beclin 1.

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Highlights

- Beclin1 plays an essential role in mediating autophagy in mammalian cells.
- Gain and loss of function of Beclin1 affect autophagy and death of heart cells.
- The function of Beclin1 is inhibited by its interaction with Bcl-2.
- Mst1 phosphorylates Beclin1 and increases Beclin1-Bcl-2 interaction.

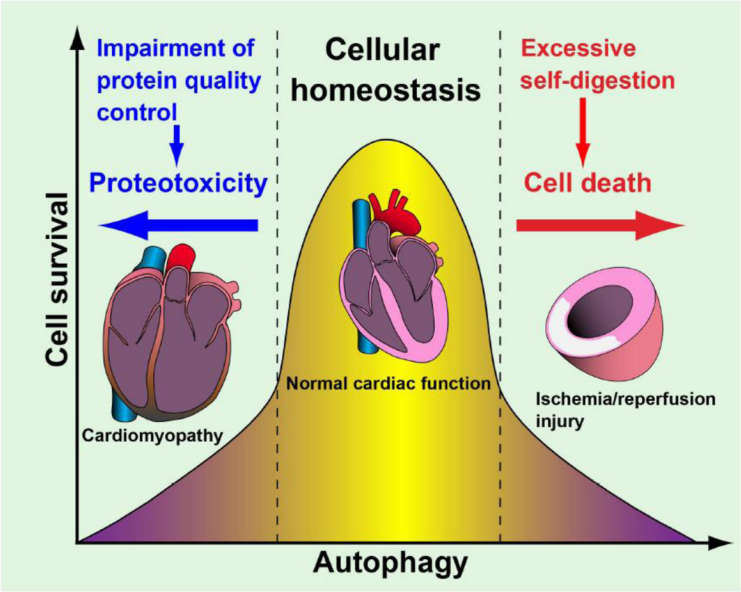

Figure 1.

Schematic representations of the relationship between the level of autophagy and heart diseases. Maintenance of physiological levels of autophagy is indispensable for normal homeostasis in cardiomyocytes. The absence of autophagy exacerbates proteotoxicity in the cells by suppressing protein quality control, which, in turn, causes the death of cardiomyocytes. However, excessive levels of autophagy also induce cell death, presumably via undue self-digestion. Thus, autophagy plays both protective and detrimental roles in the heart.

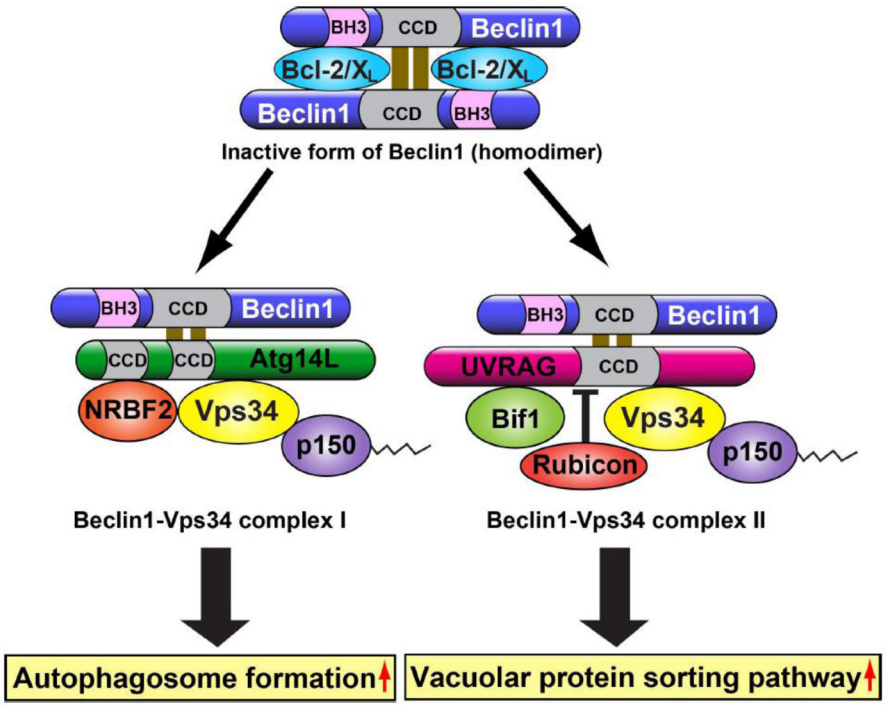

Figure 2.

Model of Beclin 1-Vps34 complexes. *Top*: Homodimerized form of Beclin 1 bridged by Bcl-2/X_L is inactive and cannot promote autophagosome formation. *Lower left*: Beclin 1-Vps34 complex I is composed of Beclin 1-Atg14 heterodimer, Vps34, p150 and NRBF2. This type of class III PI3K complex plays critical roles in both initiation and maturation of autophagosome formation. *Lower right*: Beclin 1-Vps34 complex II is composed of Beclin 1-UVRAG heterodimer, Vps34, p150 and Bif1. This type of class III PI3K complex functions in endocytic trafficking and is inhibited by Rubicon.

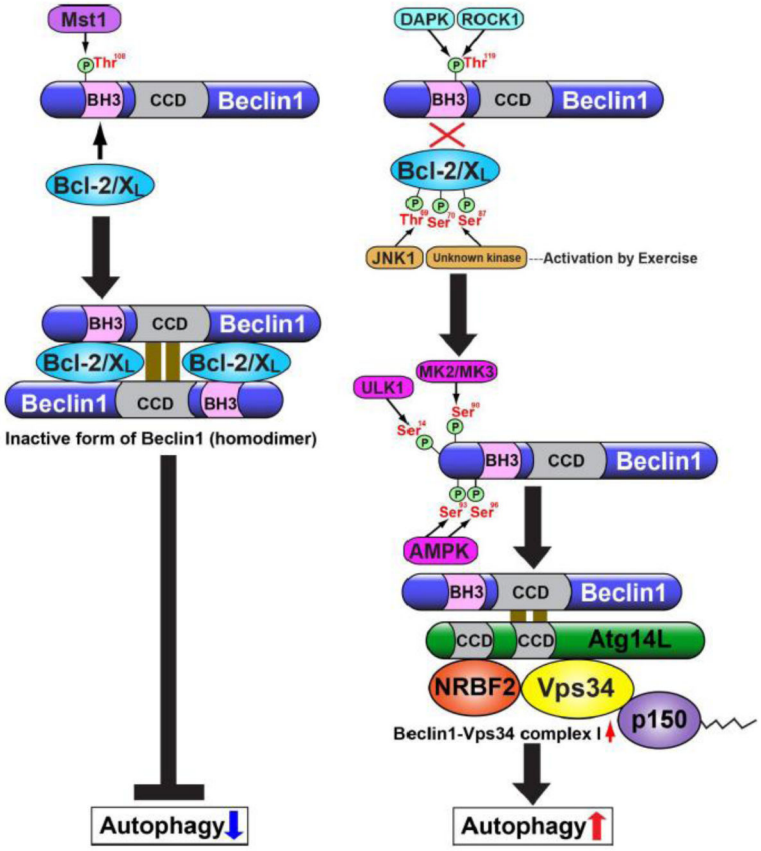

Figure 3.

Mechanisms of regulation of Beclin 1-Bcl-2/X_L interaction. *Left*: The Beclin 1-Bcl-2/X_L interaction is regulated by Mst1, which phosphorylates Beclin 1 at Thr^{108}. Beclin 1-Bcl-2/X_L binding stabilizes Beclin 1 homodimer and suppresses Vps34 kinase activity, thereby inhibiting autophagosome formation. *Right*: Conversely, phosphorylation of Beclin 1 at Thr^{119} by DAPK or ROCK1 promotes dissociation of Beclin 1 from Bcl-2/X_L. Phosphorylation of Bcl-2/X_L at Thr^{69}, Ser^{79} and Ser^{87} in the loop domain also facilitates Beclin 1-Bcl-2/X_L dissociation. Protein kinases phosphorylating the N-terminal region of Beclin 1, including MK2/MK3, ULK1 and AMPK, promote the formation of Beclin 1-Atg14L heterodimer, which results in activation of Vps34 kinase and consequent stimulation of autophagosome formation.

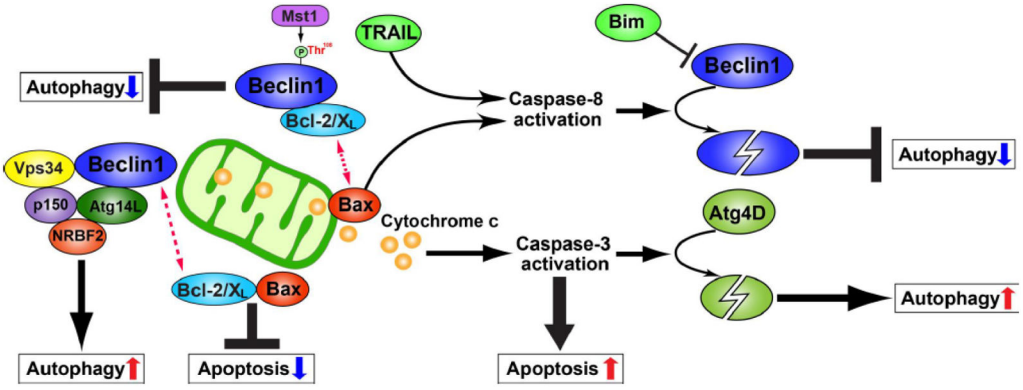

Figure 4.

Cross-talk between apoptosis and autophagy via Beclin 1-mediated signaling. Under basal condition, Beclin 1 forms Beclin 1-Vps34 complex I to induce autophagy. Concurrently, Bcl-2/X_L binds to Bax to prevent the activation of mitochondria-mediated pro-apoptotic signaling. During exposure to cellular stress, Mst1-mediated formation of Beclin 1–Bcl-2/X_L complexes suppresses autophagy. Under these conditions, the amount of free Bax increases in the cytoplasm because of increased Bax-Bcl-2/X_L dissociation. Unbound Bax translocates to the mitochondrial membrane, where it promotes cytochrome c release, which, in turn, induces apoptosis by activating caspase-3. Activated caspase-8 cleaves Beclin 1, preventing Beclin 1-mediated autophagy induction. In contrast, a fragment of Atg4D generated by caspase-3-mediated cleavage enhances autophagy activity.