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Molecular Mechanisms of Autophagy in the Cardiovascular System

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Abstract

Autophagy is a catabolic recycling pathway triggered by various intra- or extracellular stimuli that is conserved from yeast to mammals. During autophagy diverse cytosolic constituents are enveloped by double-membrane vesicles, autophagosomes, which later fuse with lysosomes or the vacuole in order to degrade their cargo. Dysregulation in autophagy is associated with a diverse range of pathologies including cardiovascular disease, the leading cause of death in the world. As such, there is great interest in identifying novel mechanisms that govern the cardiovascular response to disease-related stress. First described in failing hearts, autophagy within the cardiovascular system has been widely characterized in cardiomyocytes, cardiac fibroblasts, endothelial cells and vascular smooth muscle cells. In all cases, a window of optimal autophagic activity appears to be critical to the maintenance of cardiovascular homeostasis and function; excessive or insufficient levels of autophagic flux can each contribute to heart disease pathogenesis. Here we review the molecular mechanisms that govern autophagosome formation and analyze the link between autophagy and cardiovascular disease.

Keywords

atherosclerosis; autophagy; blood vessels; cardiac hypertrophy; cardiomyocyte; cardiovascular diseases; cardiovascular system; cell signaling; endothelial cell; fibroblast; heart; heart failure; myocardial infarction; vascular smooth muscle

Introduction

Autophagy is a key catabolic process for cell survival against different types of stress. During macroautophagy the cell carries out the sequestration of different substrates such as invading pathogens, proteins, lipids, or even damaged or superfluous organelles inside

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double-membrane vesicles termed autophagosomes. In order to degrade their cargo, autophagosomes fuse with the lysosome/vacuole, which contains various hydrolases able to break down the sequestered substrates. Following this event, the basic components obtained from cargo degradation are released into the cytoplasm for recycling.[2] Autophagy is triggered by different stimuli such as hypoxia,[3] oxidative stress,[4] pathogen infection,[5] endoplasmic reticulum (ER) stress,[6] and most notably nutrient starvation.

Autophagy can be divided into three main types: Macroautophagy, microautophagy and chaperone-mediated autophagy. Whereas microautophagy and macroautophagy can be selective or nonselective processes found in yeast and higher eukaryotes, chaperone-mediated autophagy is a selective process that has only been described in mammalian cells. During chaperone-mediated autophagy, specific protein substrates containing the amino acid sequence KFERQ are recognized by chaperones, unfolded and translocated into the lysosome through the lysosomal membrane protein LAMP2A (Figure 1A). In microautophagy uptake also occurs directly at the limiting membrane of the lysosome/vacuole. In this case, however, the process operates by directly sequestering the substrates through invagination of the vacuole/lysosome membrane.[7] Of the three types mentioned, macroautophagy is clearly the most well studied process (Figure 1B). Multiple autophagy-related (*ATG*) genes and proteins have been described as being involved in the different stages of autophagy, comprising what is now known as the core autophagy machinery that is required for autophagosome formation, and additional proteins that act in making the process selective, or in stages other than autophagosome biogenesis. Accordingly, macroautophagy (hereafter referred to as autophagy) can be dissected into different steps based on the proteins involved, including: Induction, nucleation of the autophagosome precursor (termed the phagophore), membrane expansion and maturation of the autophagosome, fusion with the lysosome/vacuole, and recycling of the degraded cargo.

Autophagy induction in yeast

During growth in nutrient-rich conditions autophagy activity is kept to a minimum by different nutrient sensing pathways including those regulated by the target of rapamycin (TOR) kinase and cAMP-dependent protein kinase A (PKA). The ability of TOR to sense nutrient levels, in particular amino acids, makes it a critical negative regulator of autophagy. The rapamycin-sensitive TOR complex 1 (TORC1) inhibits autophagy in part by preventing the activation of the Atg1 kinase complex.[8] In yeast the Atg1 kinase complex is formed by the Ser/Thr kinase Atg1, the regulatory subunit protein Atg13, and the Atg17-Atg31-Atg29 complex, which is thought to function as a scaffold.[8,9] Although specific substrates of Atg1 may remain to be discovered, the Atg1 kinase complex plays an essential role in autophagy induction by recruiting other Atg proteins to what is known as the phagophore assembly site (PAS), a perivacuolar location found in yeast that is proposed to be the organizing center for phagophore formation.[10-13] Upon nutrient starvation or rapamycin treatment TORC1 activity is inhibited and Atg13 is rapidly but partially dephosphorylated leading to the activation of Atg1.[14] Autophosphorylation of Atg1 within its activation loop is also important for activating its kinase activity and inducing autophagy (Figure 2A).[15] Atg13 dephosphorylation was linked to an increased interaction with Atg1 leading to a model in which starvation increased affinity between the two proteins; however, recent data as well as

interaction studies in other model organisms support the idea that Atg1 and Atg13 interact independently of nutrient conditions.[16] In addition to Atg13, TORC1 may also inhibit autophagy by directly phosphorylating Atg1.[17]

PKA is also a negative regulator of autophagy. PKA suppresses autophagy by phosphorylating both Atg1 and Atg13.[18-20] While the two pathways target similar proteins, TOR and PKA seem to work largely independent from one another by targeting different phosphorylation sites.[20] Other nutrient sensing kinases involved in autophagy induction are Snf1 and Gcn2. While the former corresponds to the yeast homolog of 5'-AMP-activated protein kinase (AMPK), which will be discussed below, Gcn2 promotes autophagy during amino acid starvation by phosphorylating Sui2/eIF2α. Phosphorylation of Sui2 blocks general protein synthesis and specifically activates the translation of the transcription factor Gcn4, which in turn induces the transcription of various *ATG* genes.[21, 22]

Autophagy induction in mammals

In contrast to yeast, mammalian cells have multiple Atg1 homologs, and the ones most relevant to autophagy are ULK1 (unc-51 like autophagy activating kinase 1) and ULK2.[23, 24] Thus, in mammals the Atg1 kinase complex is known as the ULK kinase complex and is formed by ULK1/2, the mammalian homolog of Atg13 (ATG13), the functional homolog of Atg17 (RB1CC1) and the ATG13 stabilizing protein ATG101, which has no yeast counterpart. All members of the ULK kinase complex are required for autophagy induction in mammalian cells.[25-27] As mentioned above, in mammalian cells the interaction between the members of the ULK kinase complex does not depend on nutrient conditions.[28] While some studies indicate that ATG13 mediates the interaction between RB1CC1 and ULK,[26] others have reported that all members of the complex can interact independently.[28] Similar to the yeast Atg1 complex, regulation of the ULK kinase complex depends on MTORC1. During nutrient-rich conditions MTORC1 interacts directly with ULK1 through the scaffold protein RPTOR and inhibits its kinase activity by phosphorylating both ATG13 and ULK1/2.[26, 29] Upon nutrient starvation or rapamycin treatment MTORC1 is released from the ULK kinase complex leading to the dephosphorylation of both proteins and the activation of ULK kinase activity.[26,28,29] Once activated, ULK1 phosphorylates ATG13, RB1CC1 and itself, stabilizing its enzymatic activity and inducing the autophagic process (Figure 2B).[26,29,30]

Another protein capable of sensing energy levels that is involved in autophagy regulation is AMPK. Through the upstream kinase STK11/LKB1, AMPK is able to sense decreases in the cellular ATP/AMP ratio leading to its activation and autophagy induction.[31] During glucose deprivation AMPK phosphorylates and activates the tuberous sclerosis complex, TSC1/2, which in turn inactivates the GTPase activating protein RHEB, leading to MTORC1 inhibition and the release of the ULK kinase complex (Figure 3).[32, 33] Once MTORC1 leaves the ULK complex, AMPK directly phosphorylates ULK1, stimulating its catalytic activity and inducing autophagy.[31] Interestingly, the ULK kinase complex also phosphorylates and inactivates AMPK, through a mechanism that has been described as an inhibitory feedback loop.[34]

Although functioning in part in a hormone-sensing pathway, AKT/PKB can also regulate autophagy by controlling MTORC1 activation. Upon ligand binding, dimerization, autophosphorylation and activation of INSR (insulin receptor) or IGF1R (insulin-like growth factor 1 receptor), the class I phosphoinositide 3-kinase (PI3K) is recruited to the plasma membrane and activated.[35] PI3K catalyzes the phosphorylation of phosphatidylinositol(4,5)bisphosphate (PIP_2) generating the lipid second messenger phosphatidylinositol(3,4,5)trisphosphate (PIP_3) which in turn recruits AKT to the plasma membrane where it is activated via phosphorylation by PDPK1 and MTORC2.[35,36] AKT-dependent phosphorylation of TSC2 prevents RHEB inhibition, leading to MTORC1 activation and autophagy inhibition.[37, 38] As a consequence, the tumor suppressor and lipid phosphatase PTEN can induce autophagy by dephosphorylating PIP_3 and downregulating the AKT-PI3K pathway (Figure 3).[39]

Membrane nucleation and source

Once autophagy is induced, assembly of the phagophore is initiated by membrane nucleation. As mentioned above, in yeast the PAS corresponds to the location at which several Atg proteins are recruited to assemble the phagophore. In contrast, mammalian cells lack a single defined PAS, and autophagosome formation seems to be initiated at different locations inside the cell. In both yeast and mammals the class III phosphatidylinositol 3-kinase (PtdIns3K) catalyzes the nucleation of the phagophore by generating phosphatydilinositol 3-phosphate (PtdIns3P) and inducing the recruitment of PtdIns3P binding proteins.[38] In yeast the PtdIns3K is formed by the regulatory subunit Vps15, the catalytic subunit Vps34, Vps30/Atg6, Atg14 and Atg38, all of which are essential for autophagy.[40-42] Similarly, the core mammalian PtdIns3K is composed of the Vps15 homolog PIK3R4, the Vps34 homolog PIK3C3, and the Vps30/Atg6 homolog BECN1.[38,43] While these three proteins constitute the core machinery of the mammalian PtdIns3K, distinct interactions with specific proteins lead to the formation of at least three different PtdIns3K complexes that play different roles in autophagy.[44-46] One of these complexes is formed by the interaction of the PtdIns3K core complex with the mammalian Atg14 homolog (ATG14) and AMBRA1.[47, 48] The ATG14-containing PtdIns3K complex is thought to positively regulate autophagy by promoting translocation of the complex to the phagophore and inducing the generation of PtdIns3P.[45,48,49] The other two PtdIns3K complexes contain the BECN1-interacting protein UVRAG as a common component. Whereas the PtdIns3K complex formed by UVRAG and SH3GLB1/Bif-1 promotes autophagosome formation,[49,50] the PtdIns3K complex formed by UVRAG and KIAA0226/RUBICON downregulates autophagy by impairing autophagosome maturation (Figure 4).[45, 46] Other BECN1-interacting proteins include the anti-apoptotic protein BCL2, which inhibits the PtdIns3K complex by sequestering BECN1 under nutrient-rich conditions.[51] Besides alterations in protein interactions, BECN1 post-translational modifications also regulate PtdIns3K activity. For example, BECN1 phosphorylation by DAPK promotes dissociation of BCL2 and autophagy induction.[52] ULK1-dependent phosphorylation of BECN1 activates the ATG14- and UVRAG-containing PtdIns3K complexes inducing autophagy during amino acid starvation.[53] Activation of both these PtdIns3K complexes by ULK1-mediated BECN1 phosphorylation would argue for the importance of this post-

translational modification for autophagosome induction and later maturation, and provides a link between the ULK kinase initiation complex and the membrane nucleation complex. Most recently, AMPK was described as regulating the activity of different PtdIns3K complexes by phosphorylating BECN1 and PIK3C3.[54]

Even though membrane nucleation has been established as a key step in the autophagic process, the origin of the membrane that gives rise to the phagophore–and subsequently the autophagosome–remains an open question. Different studies have described the ER, mitochondria, plasma membrane and *trans*-Golgi network as possible membrane donors (Figure 5).[55-57] Evidence supporting the ER as a possible membrane source include 3D tomography studies showing a connection between the phagophore and ER, as well as ATG14-containing PtdIns3K complex localization to the ER in order to initiate autophagosome formation.[49, 58] Generation of PtdIns3P at the ER triggers the recruitment of the PtdIns3P binding protein ZFYVE1/DFCP1 (zinc finger, FYVE domain containing 1) and one of the mammalian homologs of Atg18, WIPI2 (WD repeat domain, phosphoinositide interacting 2). Both of these proteins have been linked to autophagosome formation from a PtdIns3P-enriched ER-associated structure termed the omegasome for its Ω-like shape.[59, 60] Omegasomes have been described as platforms for autophagome formation which seem to depend on PtdIns3P, since ATG14 depletion leads to omegasome disappearance.[49] Although the role of ZFYVE1 in autophagy is not well defined, WIPI2 silencing results in accumulation of omegasome structures and failure to mature into autophagosomes, suggesting WIPI2 is involved in the transition between omegasomes and autophagosomes.[60] Other ATG proteins that have been associated with omegasomes include the ULK kinase complex, which localizes transiently to omegasomes in a PtdIns3P-dependent manner.[59, 61] The precise mechanism by which the ER gives rise to autophagosomes via an omegasome intermediate is unknown, and several questions remain to be answered regarding the conditions, specific proteins involved and the selectivity of the process (Figure 5).[62]

As mentioned above, mitochondria are another organelle that has been proposed as a membrane source for the phagophore. During starvation conditions an outer mitochondria membrane fluorescent marker colocalizes with autophagosomes; mitochondrial lipids also appear to transit to autophagosomes.[55] The same study showed that autophagosome formation during nutrient starvation is impaired in cells lacking the ER-mitochondria tethering protein MFN2 (mitofusin 2). Mitochondria-associated ER membrane (MAM), which are sites where the mitochondria and ER are in close proximity to each other, have been implicated in autophagosome formation. ATG14 and other autophagy markers localize to the MAM during starvation conditions. In MFN2-depleted cells, which are unable to tether the ER to the mitochondria, ATG14 localization to the MAM is impaired. Additionally, the omegasome protein marker ZFYVE1 localizes to the MAM upon starvation.[63] This remarkable finding opens the possibility that the functions of omegasomes and mitochondria in autophagosome formation are essentially one and the same, unified by the association between the two.

Other studies regarding the transmembrane protein Atg9 have advanced our understanding of the membrane source from which phagophores are assembled. Atg9 has been

characterized as a self-interacting protein containing 6 putative transmembrane domains, with both its carboxyl and amino termini facing the cytosol.[64, 65] In yeast, Atg9 cycles from the PAS to peripheral membranes; Atg9-containing vesicles are thought to be part of the initial membranes that will generate the phagophore.[66, 67] The Atg9-containing membrane reservoir appears to be composed of tubules and vesicle clusters formed through the ER-Golgi trafficking pathway;[66] however, Atg9 also cycles between peri-mitochondrial sites and the PAS.[68] While the precise mechanisms by which Atg9 cycling is controlled remains unknown, a number of Atg proteins are involved in the regulation of Atg9 movement. On the one hand, Atg9 anterograde transport, which is defined as movement from the peripheral sites to the PAS, depends on Atg11, Atg23 and Atg27.[12, 69, 70] On the other hand, retrograde transport, that is from the PAS to the peripheral sites, is directed by the Atg1-Atg13 complex, Atg2, Atg18 and the PtdIns3K complex.[12] In mammalian cells, nutrient starvation induces ATG9 redistribution from the *trans*-Golgi network to phagophores. Both ULK1 silencing and PtdIns3K inhibition block ATG9 trafficking to phagophores, suggesting both complexes are involved in mammalian ATG9 cycling.[57, 71] The MAPK pathway is also implicated in mammalian ATG9 traffic, SUPT20H/FAM48A/p38IP [suppressor of Ty 20 homolog (S. cerevisiae)] interacts with ATG9 and induces its redistribution leading to autophagy activation. Conversely, binding between SUPT20H and MAPK14/p38α inhibits ATG9 interaction with SUPT20H, and autophagy.[72]

Phagophore expansion

Elongation and expansion of the phagophore membrane is a key step in the autophagic process. The Atg12–Atg5-Atg16 and Atg8 conjugation systems, two interrelated ubiquitin-like (UBL) conjugation pathways, regulate this stage in both yeast and mammals. Before being covalently linked to their final substrates, both Atg12 and Atg8 go through an activation and conjugation reaction, triggered by an E1-like and an E2-like enzyme, respectively. In the Atg12–Atg5-Atg16 system, Atg12 is first activated in an ATP-dependent manner by the E1-like activating enzyme Atg7, forming a thioester bond between the two proteins.[73, 74] Following this event, Atg12 is transferred to the E2-like conjugating enzyme Atg10, generating the Atg12–Atg10 intermediate through the formation of another thioester bond.[75] Finally, Atg12 is covalently attached to a specific lysine residue on Atg5 in a process that, unlike ubiquitination, seems to be constitutive, irreversible and does not require an E3-like ligase enzyme.[76, 77] Further interaction between Atg12–Atg5 and Atg16 leads to the formation of the Atg12–Atg5-Atg16 complex. Unlike Atg12, Atg16 is not covalently bound to Atg5 and is able to self-interact when bound to Atg12–Atg5 forming a large multimeric protein complex.[78-80] The Atg12–Atg5-Atg16 complex is essential for autophagy and localizes to the phagophore.[79, 81, 82] In yeast the second UBL conjugation system catalyzes the lipidation of Atg8 by covalently linking it to phosphatidylethanolamine (PE). The first event in this process corresponds to the cleavage of the carboxyl terminus of Atg8 by the cysteine protease Atg4, exposing a glycine residue.[83, 84] In the next step, Atg7, again working as an E1-like enzyme, activates Atg8.[85] The activated protein is then conjugated to the E2-like enzyme Atg3, before finally being linked to PE through an amide bond.[85] Different studies have proposed that the E3-like enzyme that facilitates the Atg8–PE linkage is the Atg12–Atg5-Atg16 complex.[86-89] While in its conjugated form, Atg8 is bound

to both sides of the autophagosome membrane and thus its N terminus GFP-tagged form is widely used as an autophagosome marker.[90, 91] However, Atg8 lipidation is a reversible process since Atg8–PE bound to the external autophagosome membrane can be cleaved by Atg4, releasing it from the autophagosome (Figure 6).[83]

Both UBL conjugation pathways are conserved and work similarly between mammals and yeast with the specific difference being that mammalian cells have several Atg8 homologs further divided into the LC3 and GABARAP subfamilies. Even though all of the homologs go through a similar conjugation process, each subfamily works at different stages of autophagy;[92-94] the LC3 subfamily is involved in expansion of the phagophore and the GABARAP subfamily participates at a later stage in autophagosome maturation.[95]

Lysosome/vacuole fusion and recycling of the degraded cargo

The fusion of autophagosomes with lysosomes/vacuoles results in the generation of autolysosomes in higher eukaryotes and autophagic bodies in yeast. In either case, the fusion process appears to involve similar machinery that plays a role in other transport processes that terminate at these degradative organelles.[96] In yeast, this machinery includes the class C Vps/HOPS complex, the SNARE family proteins Ykt6, Vti1, Vam3 and Vam7, the small GTPase Ypt7 and the proteins Mon1 and Ccz1.[96–102] Fewer details are known in mammalian cells; however, the Ypt7 homolog RAB7 is required.[103] One difference between yeast and mammals is that there is a clear convergence between autophagy and the endocytic pathway in the latter; autophagosomes can fuse with endosomes to form amphisomes that subsequently fuse with the lysosome.[104] Once fusion occurs, the inner autophagosomal membrane and its cargo are degraded inside the lysosome/vacuole by various hydrolases. The resulting macromolecules such as amino acids that are obtained after cargo degradation are transported back into the cytoplasm for recycling. In yeast this process is regulated by protein permeases such as Atg22.[105]

Autophagy in the cardiovascular system

According to the World Health Organization, cardiovascular disease is the leading cause of mortality in the globalized world, accounting for 30% of all deaths.[106] As expected, considerable resources have gone toward understanding the nature of cardiovascular disease and to search for possible therapeutic targets. Autophagy has been widely described in the cardiovascular system,[107-112] and our understanding of the molecular machinery as described above provides the possibility for specific therapeutic intervention in treating cardiovascular disease. Autophagic activity is linked to cardiovascular development, preserving heart and vascular homeostasis, as well as in the onset and progression of several cardiovascular diseases. Interestingly, whether autophagy plays a survival role or has a deleterious effect during heart disease it is still a matter of discussion.

Autophagy and cardiovascular development

Autophagy plays a role in the regulation of mammalian cardiac development starting at a very early stage. Morpholino knockdown of *atg5*, *atg7*, and *becn1* result in abnormal heart structure, including defects in cardiac looping, abnormal chamber morphology and aberrant

valve development in zebrafish.[113] Similarly, *Atg5* knockout mice display defects in heart valve development and chamber septation indicating that autophagy regulates cardiac progenitor cell differentiation and is involved in heart development.

Autophagy in the genesis of cardiovascular diseases

Several risk factors underlie the genesis and progression of cardiovascular diseases. These factors include age, tobacco, unhealthy diet, insufficient physical activity, excessive weight/obesity, hypertension, diabetes and hyperlipidemia/hypercholesterolemia.[106] Hypertension is one of the largest contributors to the worldwide burden of cardiovascular diseases, and its prevalence is close to 30% in the world population.[114] The main mechanisms involved in the regulation of blood pressure are the sympathetic, parasympathetic, renin-angiotensin-aldosterone and antidiuretic hormone systems. Dysregulation of these systems, as well as obesity and diabetes, are associated with the genesis and development of hypertension.[115] Because most of the peptides and hormones belonging to these systems are capable of regulating autophagy, it is possible to speculate that dysregulation of autophagy could be associated with hypertension, obesity, diabetes and end organ damage (Figure 7).

Sympathetic and renin-angiotensin-aldosterone systems

While there is not a lot of information regarding the parasympathetic system and autophagy, studies involving the sympathetic system have shown that autophagy protects cells against excessive β-adrenergic stimulation.[116] Regarding the renin-angiotensin-aldosterone system, autophagy is stimulated by angiotensin II via the type 1 receptor (AGTR1) but diminished via AGTR2 in neonatal rat cardiomyocytes overexpressing either AGTR1, AGTR2 or both receptors after adenoviral transduction.[117, 118] Cardiomyocytes derived from a genetic rat model of heart hypertrophy are more susceptible to AGTR1–induced autophagy, but also show a strong reduction of autophagy via AGTR2.[117] Therefore, besides their well-known favorable hemodynamic and neurohumoral effects in the treatment of heart failure (HF), AGTR1 antagonists may also downregulate excessive autophagic cell death and consequently preserve cardiomyocytes.

Obesity

Autophagy is upregulated in adipose tissue of obese patients, correlating with the degree of obesity, visceral fat distribution, and adipocyte hypertrophy.[119] Xu *et al.* showed that the autophagosome maturation process is involved in high-fat diet (HFD)- and AKT2 knockout-induced cardiac hypertrophy and contractile dysfunction.[120] In the heart, HFD promotes the initiation and accumulation of autophagy, although it disrupts autophagosome maturation probably at the step of autophagosome-lysosome fusion. However, although AKT2 knockout does not affect the initiation of autophagy by HFD, it rescues HFD-induced disruption of the autophagosome maturation process and facilitates the transition from autophagosomes to autolysosomes, indicating a cardioprotective effect of cardiac autophagy in the presence of a HFD.[120]

Diabetes

Upon INS (insulin)-resistance, pancreatic β cells enhance their INS secretion to compensate for hyperglycemia. However, the progressive diminution of the number of pancreatic β cells, mainly due to apoptosis, and the decrease of their function leads to the development of type 2 diabetes mellitus (T2DM). In this model autophagy plays a protective role by limiting death of β cells. Upon exposure to a HFD, β-cell-specific *Atg7*-deficient mice display a decrease in the β cell number and an impairment of glucose tolerance due to a reduction in INS secretion.[121-124] Genetic ablation of *Atg7* in pancreatic β-cells results in degeneration of islets, impaired glucose tolerance, and reduced INS secretion.[121, 125] Moreover, cardiomyocytes isolated from T2DM *db/db* mice and HFD-induced obese mice exhibit reduced autophagic activity.[126,127] However, some recent reports conflict with these findings. Mellor *et al.* reported that increased myocardial autophagic flux in fructose diet-induced T2DM mice results in pathological remodeling of the heart.[128] Upregulation of autophagy was also found in human T2DM pancreatic β-cells.[129] These results suggest that in T2DM, increased autophagy may serve as a compensatory response to INS resistance by providing cellular components essential for maintaining normal cellular architecture and function.

Autophagy in cardiovascular diseases

In the heart, isolated cardiomyocytes, vascular epithelial cells and vascular smooth muscle cells, autophagy is strongly induced by physiological conditions, such as nutrient starvation.[130-135] In those conditions, autophagy is important for the turnover of organelles and protein aggregate degradation at low basal levels under normal conditions.[107,130] During conditions of cardiovascular stress, including ischemia/reperfusion (I/R) and heart failure, among others, autophagy is also activated.[107,110,112,136-139] However, whether autophagy in these contexts is beneficial or detrimental is not well defined.

Ischemia/reperfusion

During ischemia, autophagy is triggered as an adaptive mechanism providing nutrients and eliminating damaged mitochondria, which could otherwise release damaging reactive oxygen species and initiate apoptosis.[140] During mild ischemic stress, autophagy activation depends on AMPK-mediated inhibition of MTOR.[141,142] Pharmacological inhibition of autophagy increases cardiomyocyte death, indicating that autophagy functions as a pro-survival mechanism.[143] During chronic ischemia, autophagy can inhibit apoptosis and diminish tissue damage.[144] During reperfusion, cardiomyocyte autophagy is upregulated dramatically in rat,[145] rabbit,[146] and swine[144] hearts, and primary neonatal cardiomyocytes.[141,143] Cardiac autophagy triggered by reperfusion can be either adaptive or detrimental and involves BECN1 activation.[141] In cultured neonatal cardiomyocytes exposed to simulated I/R, inhibition of autophagy with 3-methyladenine enhances cell viability.[143] In contrast, other studies describe protective actions of autophagy in simulated I/R.[147,148] One possible explanation to these discrepancies is the dual role of BECN1 during I/R. While BECN1 is essential for initiation of autophagy, it can also inhibit vesicle processing late in the autophagic cascade, leading to cell death.[149] Thus, BECN1 abundance

can be an important determinant of autophagic activity, ensuring either survival or triggering cell death during I/R.[150]

Heart failure

The initial response of the heart to several stresses is hypertrophy.[111] If the stresses persist, a pathological hypertrophy is developed followed by heart failure.[151] In a model of pressure overload, the degree of autophagic activity correlates with the magnitude of hypertrophic growth and the rate of transition to HF.[152] Cardiomyocyte-specific overexpression of BECN1 amplifies the pathological remodeling response.[152,153] Conversely, *BECN1* haploinsufficiency partially rescues HF.[152] These data suggest that autophagy can be maladaptive under conditions of severe pressure overload. Analysis of human samples has supported additional evidence that autophagic cell death contributes to HF pathogenesis;[154,155] however, complete abrogation of autophagy is similarly maladaptive and can accelerate the progression to HF. For example, inactivation of ATG5 in adult heart is sufficient to trigger rapid-onset HF,[116] consistent with the notion that basal levels of cardiomyocyte autophagy are required for cellular proteostasis. Thus, whether elevated autophagy observed in human failing heart promotes cell death and contributes to the progression of heart failure, or if it represents an adaptive response of the heart to promote survival remains to be elucidated. Given these considerations, we propose a model in which titration of cardiomyocyte autophagy within an optimal, adaptive zone is a therapeutic goal of interest (Figure 8).[109]

Conclusions and perspectives

As one of the major catabolic pathways in the cell, autophagy has become a very important focus of research in a diverse range of biological fields. Autophagy is essential in normal cardiovascular homeostasis. However, alterations in autophagic flux are seen in all forms of cardiovascular diseases. In some instances, that response is beneficial; in other cases, it is maladaptive, promoting disease progression. Despite all knowledge accumulated so far, a substantial amount of work must be done to clarify the real contribution of autophagy in cardiovascular systems. This knowledge will not only help develop our basic understanding on how and to what end autophagy is activated, but may also give rise to potential drug treatment for several diseases. To this end, we must advance our current comprehension of the autophagic pathway and its various modulators. We are optimistic that in the near future pharmacological targeting of autophagy will emerge as a therapeutic alternative to treat cardiovascular diseases.

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Abbreviations

AGEs	advanced glycation endproducts

Atg	autophagy related
ER	endoplasmic reticulum
HF	heart failure
HFD	high-fat diet
I/R	ischemia/reperfusion
MAM	mitochondria-associated ER membrane
PAS	phagophore assembly site
PE	phosphatidylethanolamine
PI3K	phosphoinositide 3-kinase
PtdIns3K	phosphatidylinositol 3-kinase
PtdIns3P	phosphatydilinositol 3-phosphate
T2DM	type 2 diabetes mellitus
UBL	ubiquitin-like

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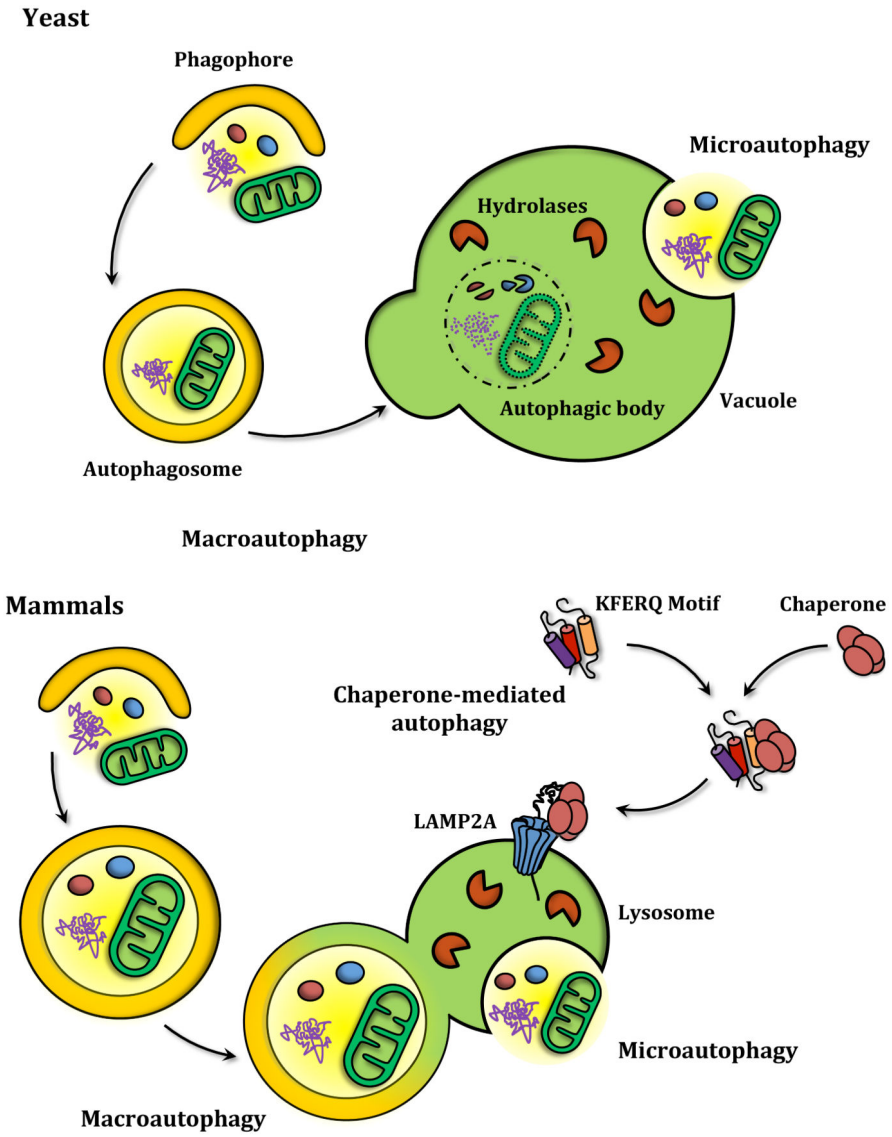

Figure 1. The three main types of autophagy

(A) Yeast cells carry out both macroautophagy and microautophagy. While macroautophagy consists of the bulk degradation of cytoplasmic material that is sequestered inside double-membrane autophagosomes that then fuse with the vacuole, microautophagy works by directly taking up the substrates through invagination of the vacuole. **(B)** In mammals along with microautophagy and macroautophagy, chaperone-mediated autophagy enables the degradation of specific protein substrates that contain a KFERQ motif that is recognized by chaperones that mediate the translocation of the protein into the lysosome through LAMP2A.

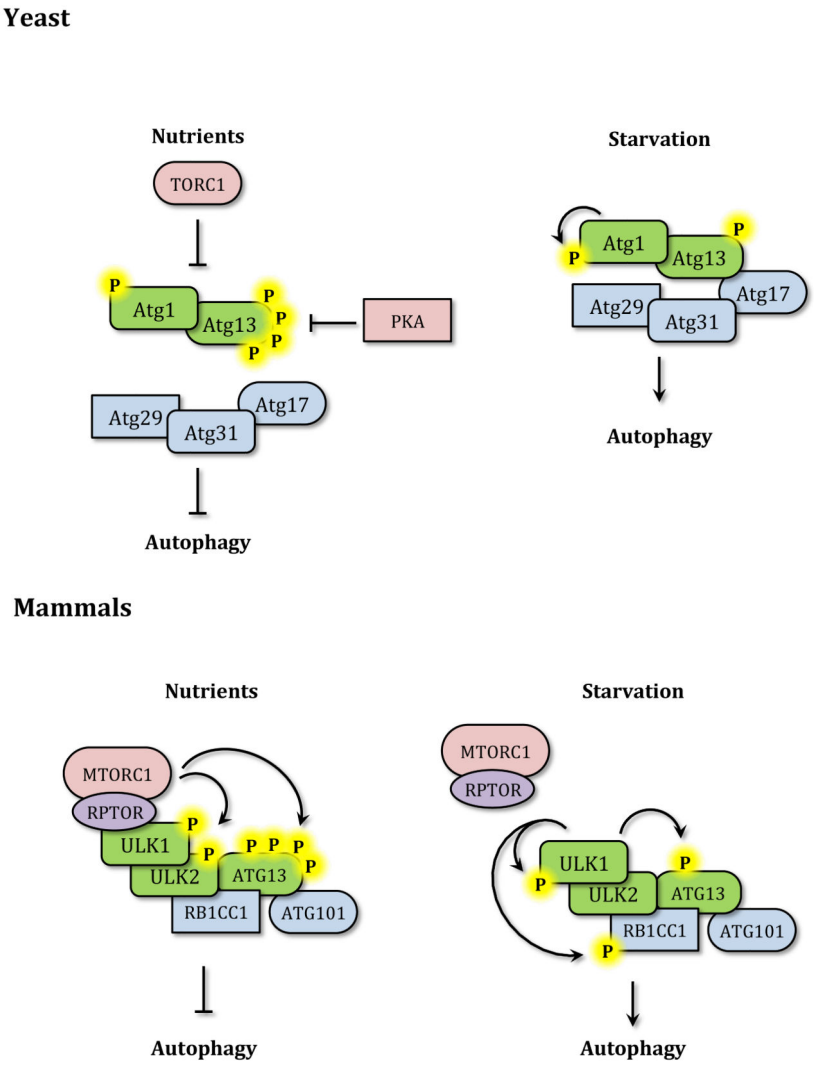

Figure 2. Autophagy induction

(A) In yeast in nutrient-rich conditions TORC1 and PKA inhibit autophagy by phosphorylating Atg1 and Atg13. During starvation the Atg1 kinase complex is no longer repressed, Atg13 is partially dephosphorylated and Atg1 is activated. Atg1 then phosphorylates itself and other targets to induce autophagy. **(B)** In mammals in nutrient-rich conditions MTORC1 directly binds ULK1 through RPTOR and inhibits ULK1/2 and ATG13 by phosphorylation. Upon starvation MTORC1 dissociates from the ULK1 kinase complex, allowing ATG13 dephosphorylation and activating ULK1/2 that then phosphorylates members of the complex and other targets to induce autophagy.

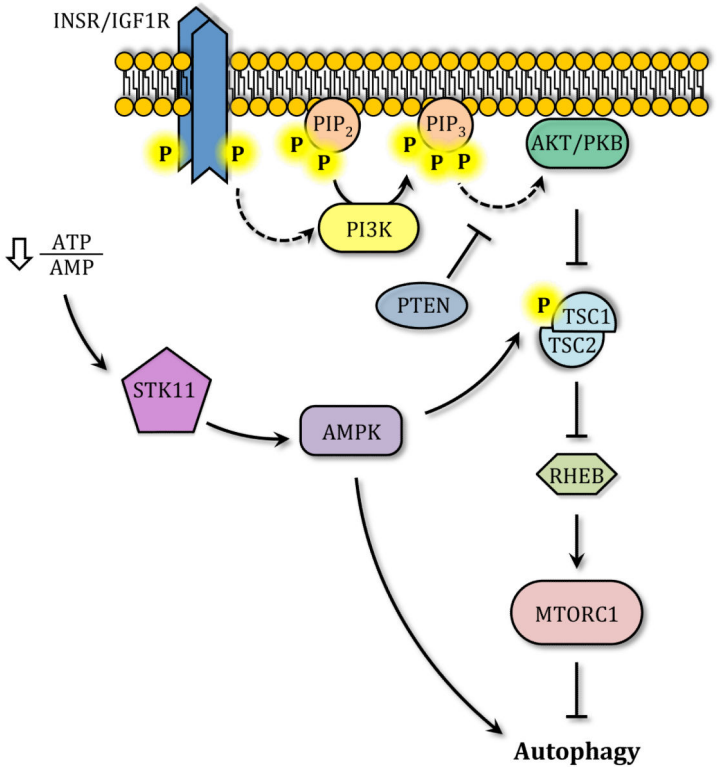

Figure 3. Autophagy regulation

Through STK11/LKB1, AMPK senses decreases in the ATP/AMP ratio and phosphorylates TSC1-TSC2, which then targets RHEB, leading to MTORC1 inhibition and autophagy activation. INSR/IGF1R triggers the activation of the class I PI3K, inducing the formation of phosphatidylinositol(3,4,5)triphosphate (PIP_3) and AKT/PKB activation; AKT can inhibit TSC1/TSC2, blocking autophagy. PTEN works as a PIP_3 phosphatase generating phosphatidylinositol(4,5)bisphosphate (PIP_2) and inducing autophagy.

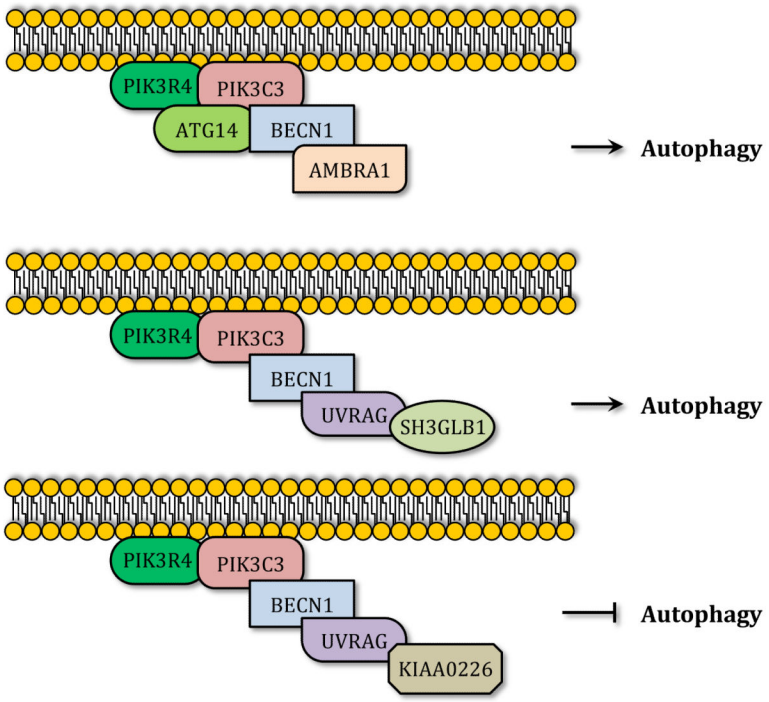

Figure 4. Class III PtdIns3K complexes

Three class III PtdIns3K complexes can be observed in mammals. All of them require PIK3C3/VPS34, PIK3R4/VPS15 and BECN1. Specific subunits regulate the function of the different complexes. Binding of ATG14 and AMBRA1 leads to autophagy induction. UVRAG and SH3GLB1 binding also activates autophagy, whereas binding to KIAA0226/RUBICON inhibits autophagosome maturation.

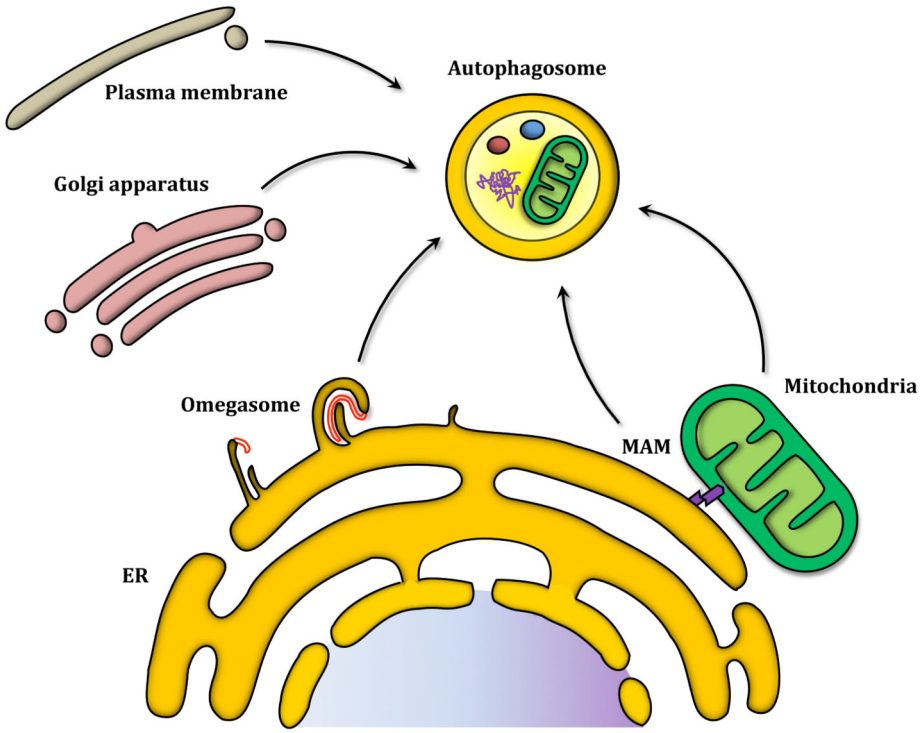

Figure 5. Autophagosomes have a diverse range of potential membrane sources

The *trans*-Golgi Network, mitochondria, mitochondrial associated membrane and ER have been postulated as membrane donors. Omegasomes have been described as the ER structures that work as a platform for autophagosome formation. The phagophore (shown in red) elongates and engulfs part of a cisternae before it buds off the ER and becomes an autophagosome.

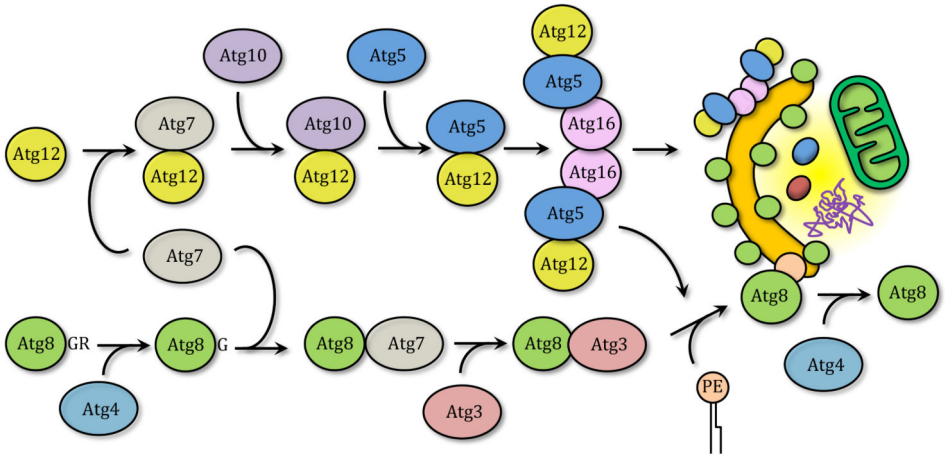

Figure 6. Two ubiquitin-like conjugation systems

Atg8 and Atg12 go through subsequent activation, mediated by Atg7 and conjugation mediated by Atg10 and Atg3, respectively, before covalently binding to PE in the case of Atg8, and Atg5 in the case of Atg12. Atg8–PE binds both the inner and outer membrane of the autophagosome, but can be deconjugated by Atg4, the same protein that removes the C-terminal arginine initially present at the Atg8 C terminus. Atg12–Atg5 bind Atg16 creating a large multimeric complex that locates to the phagophore and enhances Atg8 lipidation and membrane expansion.

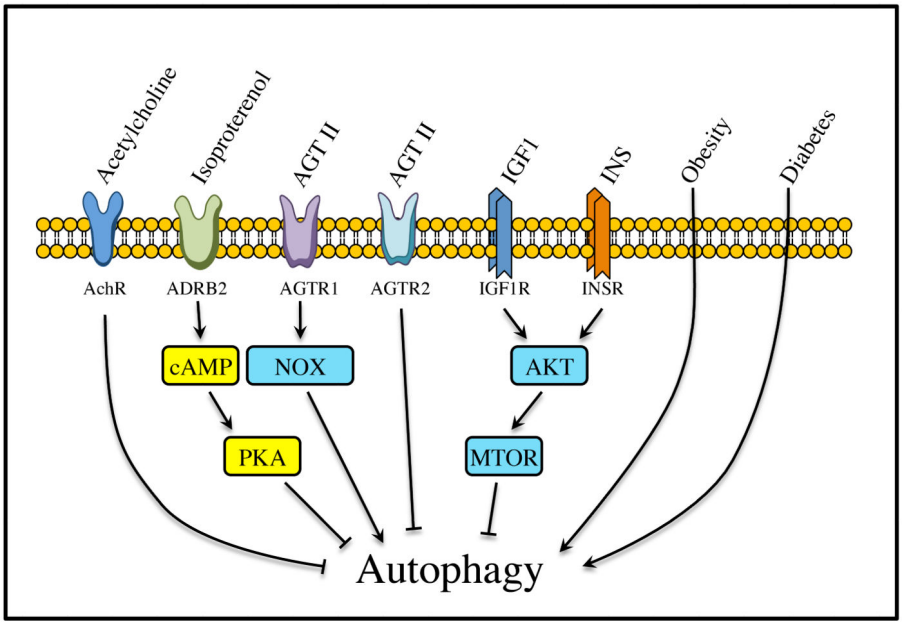

Figure 7. Autophagy regulators in cardiomyocytes

A wide variety of stimuli can regulate autophagy in cardiomyocytes. Some of them are autophagy activators and are associated with cardiovascular diseases. However, acetylcholine, catecholamines, aging, IGF1 and INS/insulin are capable of inhibiting autophagy. INS and IGF1 are well known cardioprotective agents. AchR, acetylcholine receptor; AGT II, angiotensin II.

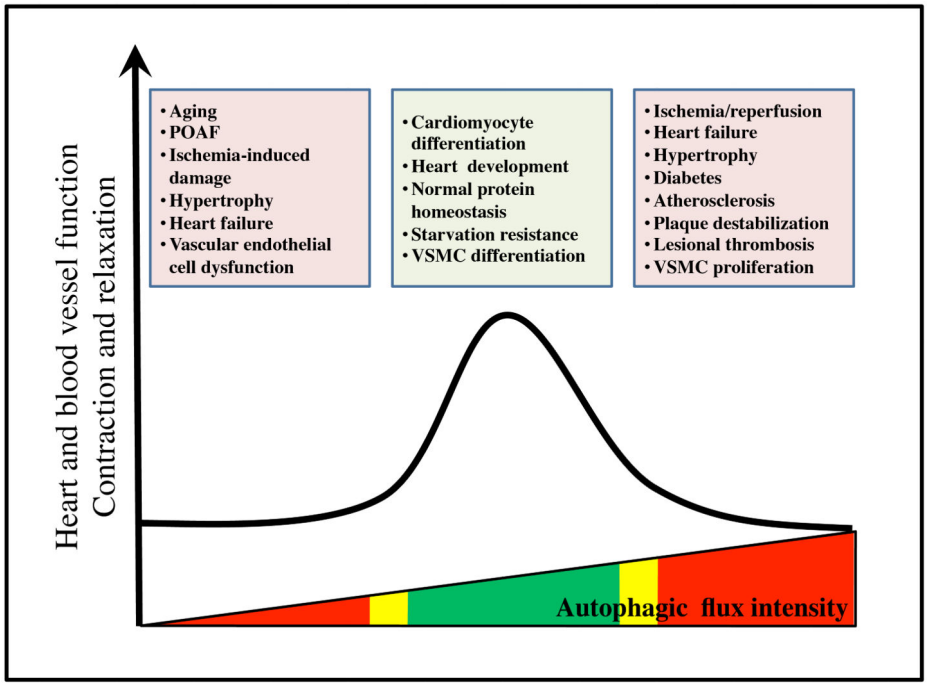

Figure 8. Autophagy in cardiovascular diseases

The relationship between autophagy and cardiovascular diseases is complex. Although basal autophagy is critical to maintain cell homeostasis, both increases and decreases in autophagy to an excessive degree can induce alterations in normal heart and blood vessel functions. In ischemia/reperfusion, heart failure, hypertrophy, diabetes, atherosclerosis, plaque destabilization, lesional thrombosis and vascular smooth muscle cell (VSMC) proliferation, autophagic flux is abnormally elevated, contributing to cardiac and vessel dysfunction. In *Atg5* and *Becn1* knockout animals and during aging, autophagic activity is decreased, perturbing cellular homeostasis and contributing to cardiovascular diseases, such as post-operative atrial fibrillation (POAF), ischemia-induced damage, hypertrophy, heart failure, and vascular endothelial cell dysfunction.