ESC European Society of Cardiology

Cardiovascular Research (2022) **118**, 934–950
doi:10.1093/cvr/cvab158

INVITED REVIEW

The role of autophagy in cardiovascular pathology

Damián Gatica [1], Mario Chiong[2], Sergio Lavandero [2,3,4], and Daniel J. Klionsky [1]*

[1]Department of Molecular, Cellular and Developmental Biology, Life Sciences Institute, University of Michigan, 210 Washtenaw Ave, Ann Arbor, MI 48109-2216, USA; [2]Department of Biochemistry and Molecular Biology, Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad de Medicina, Universidad de Chile, Olivos 1007, Independencia, Santiago 8380492, Chile; [3]Corporación Centro de Estudios Científicos de las Enfermedades Crónicas (CECEC), 926 JF Gonzalez, Santiago 7860201, Chile; and [4]Department of Internal Medicine (Cardiology Division), University of Texas Southwestern Medical Center, 6000 Harry Hines Blvd, Dallas, TX 75390-8573, USA

Received 29 January 2021; editorial decision 13 April 2021; accepted 30 April 2021; online publish-ahead-of-print 6 May 2021

Abstract Macroautophagy/autophagy is a conserved catabolic recycling pathway in which cytoplasmic components are sequestered, degraded, and recycled to survive various stress conditions. Autophagy dysregulation has been observed and linked with the development and progression of several pathologies, including cardiovascular diseases, the leading cause of death in the developed world. In this review, we aim to provide a broad understanding of the different molecular factors that govern autophagy regulation and how these mechanisms are involved in the development of specific cardiovascular pathologies, including ischemic and reperfusion injury, myocardial infarction, cardiac hypertrophy, cardiac remodelling, and heart failure.

Keywords Autophagosome • Cardiomyocyte • Heart • Lysosome • Vascular

1. Introduction

Autophagy is an evolutionarily conserved catabolic recycling pathway in which different cellular components ranging from protein aggregates to entire organelles are targeted for degradation to promote cell survival under different types of stress.[1] Autophagy induction has been observed under a diverse range of physiological and pathological conditions, including hypoxia,[2] endoplasmic reticulum (ER) stress,[3,4] oxidative stress and particularly nutrient starvation. All these stimuli are also involved in cardiovascular development, metabolism and disease; thus, autophagy regulation is a relevant subject for cardiovascular pathology.

2. Macroautophagy, microautophagy, and chaperone-mediated autophagy

Autophagy is mainly divided into three main branches, macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA), each of which has been described in detail elsewhere.[5,6] Whereas some studies have shown the importance of microautophagy and CMA in the heart,[7] the main volume of research points to macroautophagy as the main autophagic branch regulating both the physiological and pathological mechanisms involved with the cardiovascular system. Thus, for the remainder of this review, macroautophagy will be referred to as autophagy. In brief, during macroautophagy, cytoplasmic components targeted for degradation are enclosed by a rapidly expanding cup-shaped membrane called the phagophore. Expansion and maturation of the phagophore lead to the sequestration of the cargo inside double-membrane vesicles (autophagosomes) that ultimately fuse with the vacuole (in yeast and plants) or lysosome (in metazoans). Once fused together, the sequestered cargo is degraded by resident vacuolar/lysosomal hydrolases, generating macromolecules that can be then transported back into the cytosol to be recycled by the cell (*Figure 1*).[1]

3. Autophagy induction

Autophagy activation is a tightly regulated process that depends on the transcriptional,[8] post-transcriptional,[8,9] and post-translational[8] regulation of several autophagy-related (*ATG*) genes and their corresponding proteins. In nutrient-rich conditions, autophagy is usually kept at low levels; however, during nutrient starvation, autophagy activity is highly upregulated.[8] Thus, autophagy regulation by nutrient availability depends on several crucial cellular energy sensors such as MTORC1, AKT/PKB, AMPK, and PRKA/PKA.

Activation of the ULK Ser/Thr kinase complex is one of the early events required for autophagy induction. The ULK complex is formed by the catalytic subunits ULK1/2,[10,11] the regulatory scaffold protein ATG13,[12,13] RB1CC1/FIP200,[14] and the stabilizing protein ATG101.[15] As part of the initiation step of the autophagy pathway, the ULK complex

*Corresponding author. Tel: +1 734 615 6556, E-mail: klionsky@umich.edu

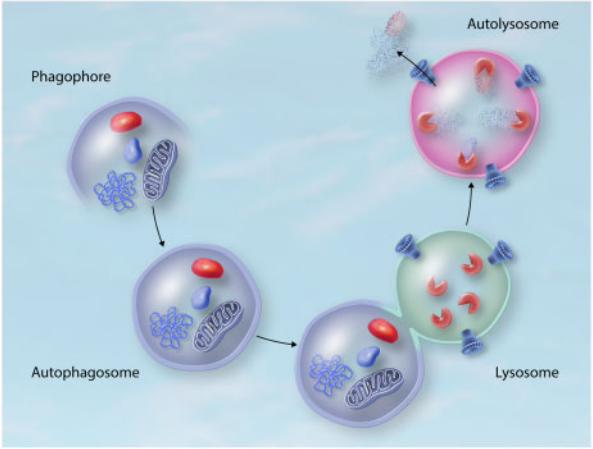

Figure 1 During macroautophagy different cytoplasmic components are enclosed by the expanding phagophore. Maturation of the phagophore results in the sequestration of the cargo inside double-membrane autophagosomes. Fusion between autophagosomes and lysosomes results in the formation of an autolysosome, where the sequestered cargo is degraded by lysosomal hydrolases. Macromolecules obtained from cargo degradation are transported back into the cytosol to be reutilized.

phosphorylates downstream ATG proteins leading to their activation and recruitment. Activation of the ULK complex during nutrient starvation conditions leads to ULK1 auto-phosphorylation[13,14] at Thr180[16] and Ser1047[17] which stabilizes its catalytic activity and in turn leads to the phosphorylation of several targets including BECN1 at Ser14[18] and Ser30,[19] ATG14 at Ser29,[20] AMBRA1 at Ser465 and Ser635,[19] ATG16L1 at Ser278,[21] RB1CC1[22] at Ser943, Ser986 and Ser1323,[19] ATG13[22] at Ser318[23] and Ser389,[19] and ATG101 at Ser11 and Ser203.[19] In nutrient-rich conditions, the ULK complex activity is inhibited by MTORC1-dependent phosphorylation of both ULK1 at Ser757[24] and ATG13 at multiple sites.[10,12,22] MTORC1 is a major sensor of the amino acid availability inside the cell, being activated by high amino acid levels through binding to an RRAG GTPase dimer[25] and the small GTPase RHEB.[26] Thus, MTORC1 is a major autophagy suppressor integrating nutrient and energy signalling into autophagy regulation. Furthermore, MTORC1 signalling has also been implicated in the transcriptional regulation of autophagy. During nutrient-rich conditions, MTORC1 phosphorylates the transcription factor TFEB at Ser211,[27] preventing its nuclear translocation and the transcription of several lysosomal[28] and *ATG* genes, including *UVRAG, WIPI, MAP1LC3B, SQSTM1, VPS11, VPS18*, and *ATG9B*.[29]

Closely related to MTORC1 signalling, the AKT/PKB pathway responds to growth factors to inhibit autophagy.[30] Activation of INSR (insulin receptor) and IGF1R (insulin like growth factor 1 receptor) triggers the activation of AKT, which in turn phosphorylates the GTPase activating protein TSC2 at Ser393 and Thr1462, preventing RHEB inhibition and leading to autophagy suppression by MTORC1.[31] Furthermore, AKT directly activates MTORC1 by phosphorylation at Ser2448.[32] AKT activation depends on the formation of phosphatidylinositol(3,4,5)trisphosphate ($PtdInsP_3$) in the plasma membrane; the phosphoinositide phosphatase PTEN is thus able to induce autophagy by dephosphorylating $PtdInsP_3$ and downregulating AKT signalling.[33] Autophagy is also directly inhibited by AKT, which can directly phosphorylate BECN1 at Ser295 and possibly Ser234, as well as ULK1 at Ser774.[16,34] Similar to MTORC1, AKT can also regulate autophagy at the transcriptional level by phosphorylating the FOXO family of transcription factors, specifically FOXO1 and FOXO3, preventing their translocation to the nucleus and inhibiting the transcription of multiple autophagy genes such as *ATG4, ATG12, BECN1, BNIP3, LC3, PIK3C3/VPS34, ULK1*, and *ULK2*.[35–37]

Whereas MTORC1 prevents autophagy activation, AMPK has been linked with autophagy induction.[24] AMPK is a heterotrimeric Ser/Thr kinase complex that can sense the energy status of the cell by binding AMP and ADP.[38,39] A decrease in the ATP:AMP ratio inside the cell results in more AMP binding to AMPK, which in turn leads to the phosphorylation of PRKAA/AMPK α-subunit at Thr172 by STK11/LBK1, resulting in AMPK activation.[40–42] Once activated, AMPK seeks to restore energy homeostasis inside the cell by upregulating catabolic pathways that can generate ATP and downregulating anabolic processes that consume energy. In this regard, AMPK plays both a direct and indirect role in autophagy regulation. Upon activation, AMPK can directly induce autophagy by phosphorylating ULK1 at Ser555[24,43] and BECN1 at Ser93 and Ser96.[44] Additionally, AMPK can also indirectly activate autophagy by preventing MTORC1-dependent inhibition of ULK1.[26,45,46] Interestingly, ULK1-dependent phosphorylation of all three subunits of AMPK has been proposed as a negative feedback loop to terminate autophagy induction.[47,48] Recently, a kinase substrate screen discovered that the cyclin-dependent kinase CCNY–CDK16 complex is a novel AMPK phosphorylation target involved in AMPK-mediated autophagy induction.[49]

PRKA/PKA is a cyclic AMP-dependent Ser/Thr protein kinase that phosphorylates LC3, preventing its recruitment to phagophores and inhibiting autophagy.[50] PRKA/PKA has also been implicated in regulating vascular network formation in endothelial cells by phosphorylating ATG16L1. PRKA/PKA-dependent phosphorylation of ATG16L1 leads to its degradation, reducing autophagy, a mechanism that could be involved in regulating the stabilization of nascent vascular endothelium.[51]

Whereas nutrient depletion constitutes the main stimulus for autophagy induction in some organisms, other types of cellular stress are also involved in autophagy regulation. These additional types of stress, relevant components of the autophagy machinery, and mechanism include the following: (i) hypoxia involving HIF1A, BNIP3, and BNIP3L, which can lead to BCL2-BECN1 dissociation;[52,53] MTORC1 inhibition, resulting in BECN1, and ATG14 phosphorylation.[54] (ii) ER stress can induce autophagy through several mechanisms involving ERN1/IRE1α and MAPK/JNK driving BCL2-BECN1 dissociation;[55–57] EIF2AK3/PERK and EIF2A/eIF2α leading to ATF4-dependent translational activation of *ATG* genes;[58–64] ATL3, CCPG1, RETREG1, RTN3, SEC62, and TEX264 receptor-dependent reticulophagy.[65,66] (iii) Oxidative stress leading to inactivation of MTORC1 and AKT, along with AMPK-dependent autophagy activation.[67–69]

4. Membrane nucleation

Generation and assembly of the phagophore require membrane nucleation by the class III phosphatidylinositol 3-kinase (PtdIns3K). This lipid kinase protein complex catalyzes the formation of phosphatidylinositol-3-phosphate (PtdIns3P), which serves as a recruitment factor for PtdIns3P-binding proteins such as those of the WIPI/WDR45 family.[70]

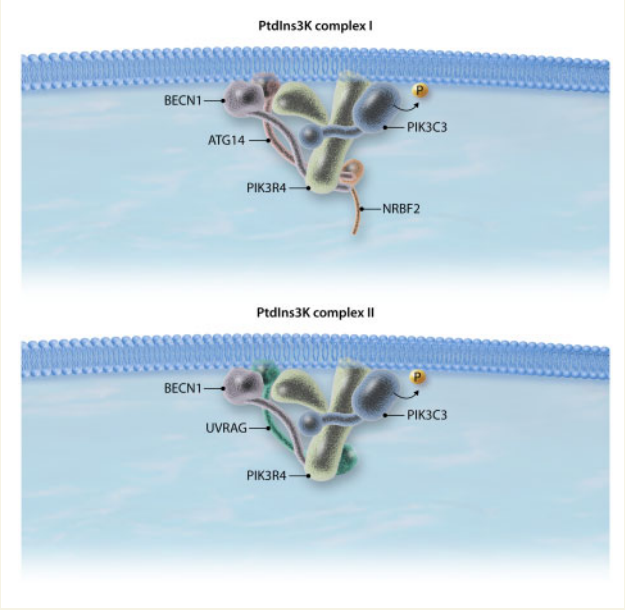

Figure 2 The proposed structure of the PtdIns3K complex I (top) and PtdIns3K complex II (bottom). Note that not all PtdIns3K complex I and complex II factors, including RUBCN and SH3GLB1, are shown.

The PtdIns3K complex is formed by several core proteins, including the catalytic subunit PIK3C3/VPS34, the regulatory subunit PIK3R4/VPS15 and BECN1.[71] Whereas these three proteins constitute the core of the PtdIns3K complex, depending on the other interacting partners, at least three other PtdIns3K subcomplexes involved in autophagy have been described.[72,73] The PtdIns3K complex I is formed by the three core proteins, in addition to the BECN1-binding proteins ATG14 and AMBRA1, and the ATG14-binding protein NRBF2.[74–76] This complex positively regulates autophagy by promoting recruitment of the PtdIns3K complex to the phagophore and inducing the generation of PtsIns3P. In this regard, ATG14 is directly associated with the ability of the PtdIns3K complex I to translocate to the phagophore;[74] AMBRA1 improves BECN1 and PIK3C3 interaction and catalytic activity;[76] and NRBF2 modulates PIK3C3 activity by promoting complex I assembly.[75] The PtdIns3K complex II is formed by the above-mentioned PtdIns3K core proteins in addition to UVRAG.[77] Although both ATG14 and UVRAG stabilize and directly interact with BECN1, their binding is mutually exclusive, generating two protein complexes with different autophagic functions[73] (*Figure* 2). Furthermore, UVRAG binding partners RUBCN/RUBICON and SH3GLB1/Bif-1 further differentiate the PtdIns3K complex II into two subpopulations with different functions. Whereas the PtdIns3K complex in which UVRAG binds SH3GLB1 stimulates autophagy by promoting the autophagosome maturation step in which autophagosomes and lysosomes fuse,[78,79] UVRAG binding to RUBCN inhibits autophagy.[73] UVRAG can be negatively regulated by MTORC1-mediated phosphorylation, which increases UVRAG and RUBCN interaction.[80]

5. Autophagosome membrane source

The membrane source from which the phagophore and subsequently the autophagosome are formed have long been a source of debate. Different studies have indicated that the initial phagophore membrane could originate from the ER,[81] *trans*-Golgi,[82] mitochondria,[83] endosomes,[84] or the plasma membrane.[85] ATG9A is the only essential transmembrane core autophagy protein, and it plays a key role in phagophore expansion,[82] in a process regulated by the adaptor complexes AP1, AP2,[86,87] and AP4.[88] In turn, AP1/2 binding is regulated by the phosphorylation of the ATG9A N terminus by SRC during nutrient-rich conditions, and ULK1 during stress.[86] Recently, the ATG9A structure has been solved, providing insight into its ability to bend and bind highly curved membranes, consistent with its role as a membrane transporter.[89] Furthermore, the ATG9A structure also revealed a possible lipid scramblase activity, which could control autophagosome size.[90] The ATG9A C terminus is responsible for binding ATG2A,[89] which works as a funnel, tethering the ER membrane to the growing phagophore and mediating lipid transfer from the ER.[91,92]

In addition to ATG9A-containing vesicles, the ER-Golgi intermediate compartment (ERGIC) has also been proposed as another possible membrane source for phagophore formation.[93] During nutrient-rich conditions coat protein complex II (COPII) vesicles travel from the ER to the Golgi as part of the secretory pathway. However, when nutrients are depleted and the secretory pathway is inhibited, COPII vesicles are repurposed for phagophore formation. During this process, the PtdIns3K complex I is recruited to the ERGIC, leading to the budding of specialized COPII vesicles that serve as a platform for LC3 lipidation, an essential step in phagophore expansion and later autophagosome maturation.[93,94] This process is regulated by ULK1, which during nutrient starvation phosphorylates the COPII essential protein subunit SEC23B, preventing its proteasomal degradation and leading to SEC23B accumulation and the generation of autophagic COPII vesicles from the ERGIC.[95]

Consistent with the idea that the ER is a major membrane source for autophagosome formation, different microscopy and 3D tomography studies have highlighted the connection between ER cisternae and pha-gophore-autophagosome membranes.[96,97] A PtdIns3P-enriched ER-subdomain (the omegasome) is linked to autophagosome biogenesis, providing a dynamic platform for recruiting several ATG proteins.[98] Omegasome formation is dependent on PtdIns3K complex I ER localization, mediated by ATG14;[99] subsequent PtdIns3P generation recruits ZFYVE1/DFCP1[98] and WIPI/WDR45 family proteins.[70] WIPI2B is required for LC3 lipidation.[70] This process is regulated by WIPI2 recruitment and direct binding to ATG16L1, which together with ATG12 and ATG5 forms the ATG12–ATG5-ATG16L1 complex, required for efficient LC3 lipidation and phagophore expansion.[100] Other WIPI family members have also been implicated in autophagy regulation. WDR45/WIPI4 forms a complex with ATG2A, allowing the latter to tether PtdIns3P-containing vesicles to non-Ptdns3P-containing membranes.[101,102] Also, the ULK1 complex associates with omegasomes in their early stages in a PtdIns3P-dependent manner.[103]

Multiple studies have indicated that autophagosomes can form at mitochondria-associated ER membranes (MAM), specific sites where the ER and mitochondria are in close proximity to one another.[83,104] During starvation conditions, ATG14 is recruited to the MAM by the ER-resident SNARE protein STX17, which is required for complete autophagosome formation.[104] Furthermore, disrupting ER-mitochondria contacts by depleting the tethering proteins MFN2 or PACS1 impairs ATG14 recruitment and starvation-induced autophagy.[83,104] ATG2A also localizes to the MAM by binding the outer mitochondria membrane protein complex formed by TOMM70–TOMM40, which in turn is required for phagophore expansion and autophagic flux.[105] Interestingly, the omegasome

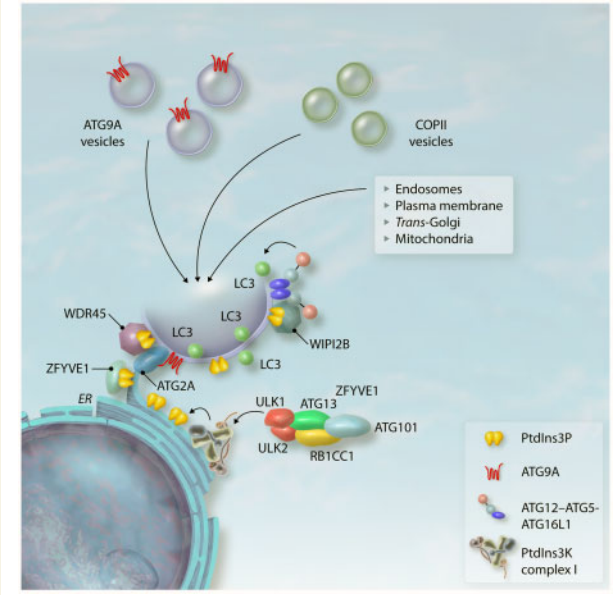

Figure 3 Translocation of the ULK complex to the ER leads to the phosphorylation and activation of the PtdIns3K complex I, which generates PtdIns3P-rich ER subdomains known as omegasomes. ZFYVE1, WDR45/WIPI4, and WIPI2B are recruited to omegasomes by binding PtdIns3P. WIPI4 binds ATG2A, which tethers the ER to the growing phagophore and transports lipids from one side to the other. ATG9A binds ATG2A, and the former moves lipids from one side of the membrane leaflet to the other, expanding the phagophore. WIPI2B recruits the ATG12–ATG5-ATG16L1 complex, inducing the lipidation of LC3 at the phagophore. Different sources provide membranes for phagophore expansion.

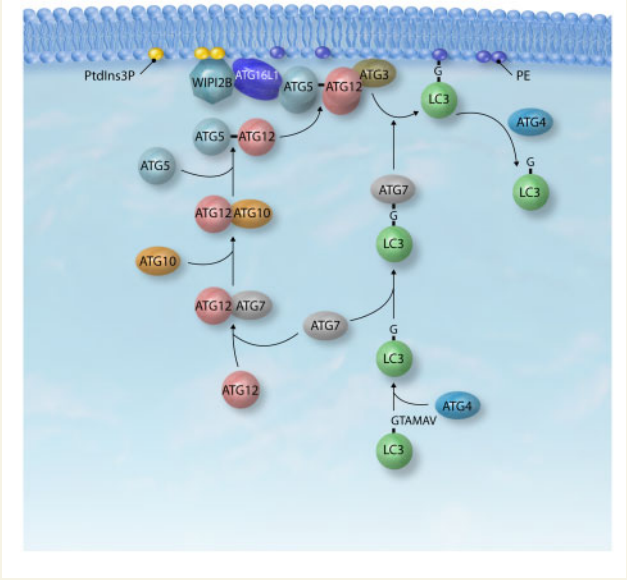

Figure 4 The ubiquitin-like proteins ATG12 and the LC3/GABARAP family go through activation by ATG7 and conjugation by ATG10 and ATG3, respectively. Covalent binding between ATG12–ATG5 leads to forming a dimeric ATG12–ATG5-ATG16L1 complex, which is recruited to the phagophore by WIPI2B, promoting the ligation of LC3 to PE. LC3-II/LC3–PE can be deconjugated by ATG4, the same protein involved in LC3 initial processing. GTAMAV, C-terminal amino acid residues; PE, phosphatidylethanolamine.

marker ZFYVE1 is also enriched in the MAM during starvation conditions, suggesting a unified system in which omegasomes and ER-mitochondria contacts are adjacent to each other.[104]

Taken together, an overall model has started to emerge in which the ER and ER-associated membranes serve as the main platform and membrane source for phagophore formation. Membranes from other organelles are very likely to contribute to autophagosome formation by generating membrane contact sites with the ER. Electron microscopy and electron tomography studies have shown phagophores making contact sites with membranes from late endosomes, Golgi, and mitochondria, sometimes even simultaneously.[106] Therefore, it is reasonable to think of the ER as an expanding membranous system across the cell, interconnecting with different organelles that supply the phagophore with their membranes (*Figure 3*).

6. Expansion of the phagophore membrane

Two interconnected ubiquitin-like conjugation systems are involved in expanding the phagophore membrane, the ATG12–ATG5-ATG16L1 complex and the lipidation of the LC3/GABARAP family (*Figure 4*). The mechanism involved in the conjugation of LC3/GABARAP to phosphatidylethanolamine (PE), involving the action of the ATG12–ATG5-ATG16L1 complex as an E3 ligase has been described in great detail elsewhere.[107–109] Binding between ATG16L1 and WIPI2 may control the targeting of the ATG12–ATG5-ATG16L1 complex and in turn the site of LC3/GABARAP lipidation.[100] ATG16L1 is also able to interact with the ULK1 complex subunit RB1CC1/FIP200, which plays a role in the recruitment of the ATG12–ATG5-ATG16L1 complex to the phagophore.[110] Recently, ATG16L1 has been proposed to bind directly to membranes;[111] thus, it seems that the localization of ATG12–ATG5-ATG16L1 depends on multiple factors, including protein and lipid interactions.

Beyond their role in the expansion and maturation of autophagosomes, when covalently bound to PE, the LC3/GABARAP family serve as interaction platforms for a diverse range of different proteins, physically linking them to the phagophore membrane. Among these LC3/GABARAP-binding proteins are various autophagy receptors that play an important role in selective autophagy, tethering the cargo targeted for degradation to the growing phagophore. These receptors include SQSTM1/p62, OPTN, TOLLIP, and NBR1, which participate in the autophagic degradation of various types of ubiquitinated cargo; BNIP3L and FUNDC1 which have been linked to mitophagy;[112] RETREG1, CCPG1, SEC62, RTN3, and TEX264, which have been connected to reticulophagy;[65] and CALCOCO2 that has been reported to induce the selective autophagic degradation of intracellular pathogens.[113] Binding between these receptor proteins and LC3/GABARAP is generally mediated by the LC3-interacting region (LIR), a distinct amino acid sequence closely resembling Trp-X-X-Leu/Ile.[109,114] A specific type of LIR termed GABARAP-interacting motif has been described, which resembles the sequence Trp/Phe-Val/Ile-X-V and shows a higher binding affinity for the

GABARAP subfamily.[115] Besides the autophagy receptor proteins, several other core components of the autophagy machinery such as ULK1, ATG13, RB1CC1,[116] BECN1, ATG14,[117] ATG4,[118] and ATG7[119] have been proposed to possess LIR sequences that allow them to interact with LC3/GABARAP and be recruited to the phagophore. Even though most of the research involving LC3/GABARAP and their interacting partners has been focused on the interface between the LIR and its corresponding docking site on the various LC3/GABARAP proteins, a recent publication has reported a novel alternative binding site in this family that binds ubiquitin-interacting motifs, opening the door for the discovery of new LC3/GABARAP-interacting partners and mechanisms of autophagy regulation.[120]

7. Lysosomal fusion and cargo degradation

In the last stages of autophagy, complete autophagosomes must fuse with lysosomes to release their cargo for degradation. This process requires a spatial approach between the two organelles that allow for membrane tethering and fusion to occur. Mature autophagosome trafficking is mediated by microtubules that serve as tracks, allowing the transport of complete autophagosomes to the perinuclear region, where lysosomes are usually located during starvation.[121,122] Once autophagosomes and lysosomes are sufficiently close, tethering and fusion are triggered by multiple factors, including RAB7 and the HOPS complex,[123,124] STX17, SNAP29, VAMP7, and VAMP8.[125–127] Specificity of the lysosome-autophagosome fusion process is provided by several different adaptor proteins; for example, the RAB7 effector protein PLEKHM1 directly interacts with the HOPS complex and LC3/GABARAP through an LIR, providing a bridge between autophagosomes and the different members of the tethering complex.[128] Once fusion is completed, the inner autophagosomal membrane is degraded inside the newly formed autolysosome, where lipases and acid hydrolases including cathepsins CTSB, CTSD, and CTSL degrade the cargo, generating essential molecules such as amino acids that can be transported back to the cytosol to be recycled by the cell. Recent studies have identified the lysosomal multi-spanning transmembrane protein SLC38A9 as a central amino acid transporter responsible for recycling amino acids from the lysosomal lumen to the cytosol.[129–131] SLC38A9 senses Arg, which plays a modulating role in the lysosomal efflux of Gln and Leu and other essential amino acids to the cytosol.[132,133] SLC38A9-dependent amino acid efflux is sensed by MTORC1,[129–133] which is activated, generating a negative feedback loop that finally inhibits autophagy.

8. Cardiac autophagy

Autophagy has a vital role in the normal and diseased heart.[134–136] Basally, cardiomyocytes display beneficial autophagy to degrade misfolded proteins and damaged organelles.[134,137] Moreover, autophagy is required for normal cardiac development.[138] Knockdown or knockout of essential autophagy genes, including *Atg5*, results in defects in cardiac morphogenesis, notably in valve development and chamber septation.[139] However, this process is altered during metabolic stresses (e.g. diabetes, lipotoxicity), ischemia/reperfusion (I/R) injury, myocardial infarction (MI), cardiac hypertrophy, cardiac remodelling, and heart failure (HF).[134–136] Because our previous review covered cardiovascular autophagy up to 2014,[134] we focus mainly on the new findings described since 2015. The main discoveries in the cardiovascular area are described in *Table 1*.

9. Mitochondrial dysfunction and mitophagy

Because of constant beating, the heart is an organ that requires a continuous high supply of ATP. The heart produces and utilizes ~6 kg ATP per day.[190] Therefore, mitochondrial metabolism and function are essential for cardiac homeostasis.[191] Mitophagy, the selective autophagic degradation of mitochondria, is an important mediator of mitochondrial quality control in cardiac myocytes. Removing dysfunctional mitochondria through mitophagy is essential for maintaining cardiomyocyte energetic and metabolic requirements.[191] In developing hearts, the MFN2–PINK1–PRKN pathway, which poly-ubiquitinates damaged mitochondria to promote mitophagy, changes the cardiac metabolism by redirecting the mitochondrial substrate preference to fatty acids.[157] Through this mechanism, foetal cardiomyocyte mitochondria are removed by PRKN-mediated mitophagy and replaced by mature adult mitochondria.[157] Cardiac mitophagy is also observed during cardiac ischemia by a complex consisting of ULK1, RAB9, RIPK1, and DNM1L.[192] Downregulation of mitophagy mediates the development of mitochondrial dysfunction and HF.[162] In this model, an isoform shift from PRKAA2/AMPKα2 to PRKAA1/AMPKα1 is observed in mouse hearts and in heart samples from HF patients.[193] BAG3, a co-chaperone of HSPA/HSP70, is also recruited to depolarized mitochondria, together with PRKN to promote mitophagy,[194] in part by regulating translation of LC3B.[195] Suppression of BAG3 in cardiac myocytes reduces autophagy flux and mitophagy.[194] Conversely, administration in the heart of BAG3 using an adeno-associated virus improves left ventricular ejection fraction (LVEF) after MI.[196] Restoration of PRKAA2 activates the PINK1–PRKN–SQSTM1 pathway that increases cardiac mitophagy associated with the improvement in mitochondrial function, removal of damaged mitochondria, decrease in reactive oxygen species (ROS) production, and cardiomyocyte apoptosis.[193]

Single-nucleotide polymorphisms in the *PRKN* are linked to blood pressure in Nigerian and Korean families, suggesting an association between mitophagy and hypertension.[197] Moreover, spermidine supplementation in the diet of salt-sensitive rats enhances cardiac mitophagy and delays the development of hypertensive heart disease.[198] Also, in a unilateral renovascular hypertension pig model, valsartan, a well-known antihypertensive drug, reduces left ventricular hypertrophy and increases mitophagy and mitochondrial biogenesis.[198] These data suggest that the increase of mitochondrial turnover decreases hypertensive-dependent cardiac remodelling.

10. Ischemia/reperfusion

Myocardial ischemia is one of the main causes of sudden cardiac death worldwide, and activation of autophagy protects cardiomyocytes from I/R injury.[134] During ischemia, autophagy is triggered as a pro-survival mechanism in response to nutrient and oxygen deprivation.[199] However, during reperfusion, autophagy has beneficial or detrimental effects depending on the experimental model and whether it involves a BECN1- or an AMPK-MTOR-dependent activation of autophagy.[144,199,200] More recent findings indicate that induction of autophagy by simultaneous administration of PT1, a specific activator of AMPK,

Table 1 Timeline describing principal findings in cardiovascular autophagy

	Year	Description
Heart	1976	First description of autophagy in cardiomyocytes[140]
	2000	First description of autophagy in hearts associating accumulation of autophagic vacuoles and cardiomyopathy in LAMP2-deficient mice[141]
	2001	Autophagic degeneration as a possible mechanism of myocardial cell death in dilated cardiomyopathy[142]
	2003	Myocytes die by multiple mechanisms in failing human hearts[143]
	2007	Cardiac autophagy is a maladaptive response to haemodynamic stress
	2007	Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMPK and BECN1 in mediating autophagy[144]
	2007	The role of autophagy in cardiomyocytes in the basal state and in response to haemodynamic stress[145]
	2008	Intracellular protein aggregation is a proximal trigger of cardiomyocyte autophagy
	2009	Markers of autophagy are downregulated in failing human heart after mechanical unloading[146]
	2010	Deacetylation of FOXO by SIRT1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes[147]
	2012	Autophagy proteins LC3B, ATG5, and ATG12 participate in quality control after mitochondrial damage and influence lifespan[148]
	2012	First description of autophagy in cardiac fibroblast assessing the role of ADRB2/beta(2)-adrenergic receptor in the regulation of cardiac fibroblast autophagy and collagen degradation[149]
	2012	Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure[150]
	2012	Impaired autophagosome clearance contributes to cardiomyocyte death in ischemia/reperfusion injury[151]
	2013	Mechanical unloading activates FOXO3 to trigger BNIP3-dependent cardiomyocyte atrophy[152]
	2013	PINK1-phosphorylated MFN2 is a PRKN receptor for culling damaged mitochondria[153]
	2013	Autophagy regulates endothelial cell processing, maturation, and secretion of VWF[154]
	2013	STK4/MST1 inhibits autophagy by promoting the interaction between BECN1 and BCL2[155]
	2014	Autophagy regulates vascular endothelial cell NOS3 and EDN1 expression induced by laminar shear stress in an *ex vivo* perfused system[156]
	2015	PRKN-mediated mitophagy directs perinatal cardiac metabolic maturation in mice[157]
	2014	PRKN-independent mitophagy requires DNM1L and maintains the integrity of mammalian heart and brain[158]
	2015	Endogenous DNM1L mediates mitochondrial autophagy and protects the heart against energy stress[159]
	2015	Interdependence of PRKN-mediated mitophagy and mitochondrial fission in adult mouse hearts[160]
	2015	SIRT7 contributes to myocardial tissue repair by maintaining the TGFB signaling pathway[161]
	2016	DNM1L-dependent mitochondrial autophagy plays a protective role against pressure overload-induced mitochondrial dysfunction and heart failure[162]
	2016	Restoration of autophagy in endothelial cells from patients with diabetes mellitus improves nitric oxide signaling[163]
	2016	Doxorubicin blocks cardiomyocyte autophagic flux by inhibiting lysosome acidification[164]
	2016	Cardioprotection and lifespan extension by the natural polyamine spermidine[165]
	2017	Endothelial-specific deletion of *Atg7* attenuates arterial thrombosis in mice[166]
	2017	Endothelial cell autophagy maintains shear stress-induced nitric oxide generation via glycolysis-dependent purinergic signaling to endothelial NOS3[167]
	2017	Autophagy is required for endothelial cell alignment and atheroprotection under physiological blood flow[168]
	2018	Endothelial autophagic flux hampers atherosclerotic lesion development[169]
	2018	BECN1-dependent autophagy protects the heart during sepsis[170]
	2018	TLR4 contributes to a myofibroblast phenotype in cardiac fibroblasts and is associated with autophagy after myocardial infarction in a mouse model[171]
	2019	Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy[172]
	2020	Downregulation of BECN1 promotes direct cardiac reprogramming[173]
Blood vessels	1997	First description of autophagy in vascular endothelial cells and cardiac endothelial cells obtained from mouse hearts after long-term exposure to non-metabolizable sugars[174]
	2001	*Porphyromonas gingivalis* traffics to autophagosomes in human coronary artery endothelial cells[175]
	2006	First description of autophagy in VSMC by evaluating the effects of IGF1 and TNF in the regulation of autophagy through MAPK/JNK and AKT pathways in human atherosclerotic vascular smooth cells[176]

Continued

Table I *Continued*

Year	Description
2011	BECN1 deficiency is associated with increased hypoxia-induced angiogenesis[177]
2015	Defective autophagy in VSMCs accelerates senescence and promotes neointima formation and atherogenesis[178]
2018	Impaired mitochondrial respiration in human carotid plaque atherosclerosis: a potential role for PINK1 in VSMC energetics[179]
2019	Altered mitochondrial quality control in ATG7-deficient VSMCs promotes enhanced apoptosis and is linked to unstable atherosclerotic plaque phenotype[180]
2019	VSMC plasticity and autophagy in dissecting aortic aneurysms[181]
2020	Defective autophagy in vascular smooth muscle cells increases passive stiffness of the mouse aortic vessel wall[182]
2019	FOXO1 inhibits autophagosome-lysosome fusion leading to endothelial autophagic-apoptosis in diabetes[183]
2019	Endothelial-specific deficiency of ATG5 attenuates ischemia-related angiogenesis[184]
2020	Mitophagy contributes to endothelial adaptation to simulated microgravity[185]
2020	Defective autophagy in vascular smooth muscle cells alters vascular reactivity of the mouse femoral artery[186]
2020	Endothelial autophagy deficiency induces IL6-dependent endothelial mesenchymal transition and organ fibrosis[187]
2020	Laminar flow inhibits the STK3–STK4 (HIPPO)–YAP1 pathway via autophagy and SIRT1-mediated deacetylation against atherosclerosis[188]
2020	Ischemia induces autophagy of endothelial cells and stimulates angiogenic effects in a hindlimb ischemia mouse model[189]

and 3HOI-BA-01, a potent inhibitor of MTOR, reduces cardiomyocyte death triggered by simulated I/R. *In vivo* administration of PT1 or 3HOI-BA-01 in a murine I/R injury model stimulates autophagy and reduces infarct size.[201] Likewise, cardiomyocyte-specific autophagy disruption with a conditional *atg7* knockout leads to myofibrillar disarray and severe contractile dysfunction and worsens the I/R injury with cardiac hypertrophy and severe cardiac fibrosis.[202] Autosis, a form of cell death due to excessive activation of autophagy, is induced in cardiomyocytes exposed to I/R.[203] This autosis is associated with RUBCN upregulation, autophagic flux attenuation, and autophagosome accumulation.[203] Furthermore, inhibition of excessive I/R-induced autophagy by trimetazidine protects the rat hearts from I/R-induced HF.[204]

11. Cardiac fibrosis

The differentiation of cardiac fibroblast to myofibroblast is one of the most important features of fibrosis.[205] Autophagy is implicated in cardiac fibrosis because autophagy inhibition blocks fibroblast-to-myofibroblast phenoconversion and inhibits myofibroblast cell migration and contractility.[206] Moreover, TGFB1 treatment of human atrial fibroblasts induces autophagy and enhances the fibrogenic response, suggesting an association between the myofibroblast phenotype and autophagy.[207] In streptozotocin-induced diabetic rats, alterations in autophagy correlate with cardiac fibrosis, indicating a potential synergistic role for autophagy in diabetic cardiac fibrosis.[208] Moreover, *in vitro* and *in vivo* studies show that inhibition of PARP1 partially decreases autophagy, abrogates cardiac fibrosis, and significantly improves cardiac function post-MI.[209] In a mouse model of lipopolysaccharide-induced sepsis, cardiac-specific overexpression of BECN1 promotes autophagy, improves cardiac function, and alleviates inflammation and fibrosis.[170]

12. Diabetic cardiomyopathy

Diabetic cardiomyopathy is a cardiovascular disease characterized by morphological, functional, and metabolic changes in the heart produced as a complication of type 2 diabetes mellitus (T2DM).[210] Excessive autophagy is observed in the right atrial appendages collected from diabetic and non-diabetic patients.[211] Similarly, animal models of T2DM show increased autophagosomes in the heart that is further increased upon chloroquine injection. Importantly, *in vitro* genetic deletion of *Becn1* in adult rat cardiomyocytes exposed to high glucose markedly inhibits autophagy and triggers apoptosis suggesting a pathological role of autophagy in the T2DM heart.[211] Moreover, excessive autophagy in T2DM induces HF aggravation after MI through defective mitophagy, associated with excessive inflammasome activation, secretion of IL18, and cell death.[212] Prevention of inflammation by NFKB blockade with pyrrolidine dithiocarbamate reduces oxidative stress and improves mitochondrial integrity, thus restoring cardiac function in T2DM.[213] In hearts from obese *db/db* T2DM mice, LC3 lipidation, SQSTM1, and phosphorylated MTOR levels are increased, but CTSD level is decreased, and very few lysosomes are detected, despite the abundance of autophagic vacuoles. In these hearts, AMPK activity and ATP content are decreased. These findings suggest that in this T2DM model, the autophagic flux is blocked at the final step.[214] In high-fat diet-induced obese mice, a failure to upregulate LC3 lipidation or to clear SQSTM1 in the heart after fasting are observed, although mRNA for *Lc3b* and *Sqstm1* are appropriately upregulated. These data suggest that hearts of diet-induced obese mice also exhibit impaired autophagy.[215]

In hearts from streptozotocin-induced type 1 diabetic mice (T1DM), diastolic dysfunction is observed,[214,216] although autophagic activity is increased, as evidenced by increases in LC3-II and CTSD, and decreased SQSTM1, and by the abundance of autophagic vacuoles and

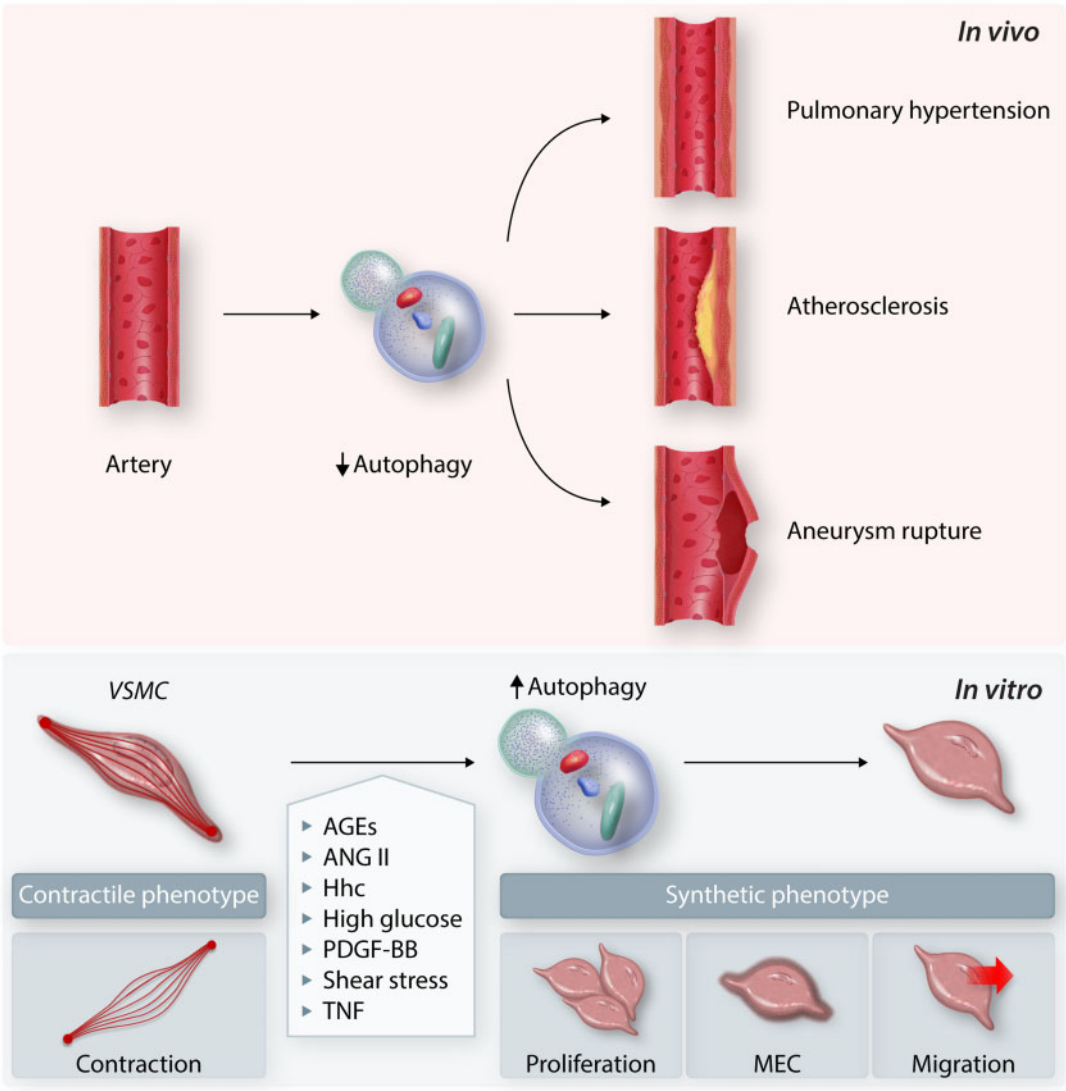

Figure 5 Role of autophagy in the development of vascular diseases using *in vivo* models and VSMC phenotypic change using *in vitro* models. AGES, advanced glycation end products; Ang II, angiotensin II; Hhc, hyperhomocysteinemia; PDGF-BB, platelet-derived growth factor-BB; TNF, tumor necrosis factor; VSMC, vascular smooth muscle cell.

lysosomes.[214] The increase of blood glucose level in T1DM induces mitochondrial damage by O-GlcNAcylation of many electron transport chain subunits and other mitochondrial proteins.[217] Inhibition of autophagic flux by chloroquine decreases autolysosomes, cardiomyocyte apoptosis, and cardiac fibrosis, but increases both *Lc3* and *Sqstm1* expression.[218] Surprisingly, mitophagy is inhibited in hearts from T1DM mice.[219] Moreover, there is substantial attenuation of cardiac damage in mice deficient for BECN1 and ATG16L1 as evidenced by an improvement in cardiac function along with decreased levels of oxidative stress, interstitial fibrosis, and cardiomyocyte apoptosis. In contrast, diabetes-induced cardiac injury is aggravated by BECN1 overexpression.[220] In the same way, administration of 1,25-dihydroxyvitamin-D3 and resveratrol improves diabetic cardiomyopathy by restoring the impaired cardiac autophagy in streptozotocin-induced diabetic rats.[221,222]

13. Heart failure

HF is a clinical syndrome due to a structural and/or functional cardiac abnormality, which results in a reduction in cardiac output and/or elevated intracardiac pressure.[223] HF affects patients ranging from those with normal (≥50%) LVEF, known as HF with preserved EF (HFpEF), to those with reduced (<40%) LVEF, known as HF with reduced EF (HFrEF).[223] All these patients display signs of HF with evidence of abnormal cardiac structure and function.[224] Epidemiological studies have shown that the incidence of HFrEF and HFpEF are almost equally distributed, but the incidence of HFpEF is increasing with time.[225] The role of autophagy is well described in HFrEF;[134] however, its role in HFpEF is poorly defined. Recently, a gene expression analysis performed on heart biopsies obtained from HFrEF, HFpEF, and control patients revealed that autophagy genes are strikingly downregulated in HFpEF patients.[226]

Table 2 Clinical trials involving autophagy in the cardiovascular system

Name of the study	Study type	Description	Clinical trial identifier
MUcociliary Clearance IN Stroke (MUCINS)	Observational	Evaluation of autophagy in respiratory tissue (nasal, tracheal, and bronchial)	NCT03884166
Transforming Growth Factor Beta Signalling in the Development of Muscle Weakness in Pulmonary Arterial Hypertension	Observational	Determination of the contribution of atrophy and autophagy to muscle wasting in PAH	NCT01847716
Intermittent Pneumatic Compression With and Without Exercise to Improve Functioning in Peripheral Artery Disease (INTERCEDE)	Interventional	Evaluation among PAD participants whether intermittent pneumatic compression therapy combined with exercise improves autophagy (LC3, LAMP2, PRKN) at 6-month follow-up, compared to exercise alone	NCT03871075
Autophagy and Venous Endothelial Function	Observational	Evaluation of endothelial function in venous samples from patients with venous insufficiency before and after treatment with autophagy enhancer spermidine	NCT04138134
Autophagy Maintains Vascular Function Through a Novel Glycolysis-linked Pathway Regulating eNOS	Interventional	Evaluation of BECN1, ATG3, ATG5, ATG7, LAMP1, LAMP2, and SQSTM1 after rhythmic handgrip exercise or chronic exercise training	NCT04200560
Autophagy, Oxidative Stress, and Hippo Signalling in Human Aortic Aneurysm	Observational	Comparison between the levels of STK3–STK4 (HIPPO) signalling and autophagy markers observed in aortic aneurysms and the levels assessed in the adjacent non-aneurysmatic aortic portions	NCT03211000
Effects of Trehalose and Polyphenols in Vasculopathic Patients	Interventional	Change of autophagy (LC3) in PAD patients after mixed supplementation of trehalose and polyphenols	NCT04061070
Effect of Hydroxychloroquine on Atrial Fibrillation Recurrence	Interventional	Recurrence rate of atrial fibrillation after radiofrequency catheter ablation, anticoagulant therapy, and hydrochloroquine treatment (200 mg, bidpo)	NCT03592823
Potential Impact of Neuroimmune and Autophagic Alterations on the Progression and Severity of Human Atherosclerotic Process	Observational	Characterization of the autophagic process and correlation with neural modulations of the stability/instability plaque	NCT03922698

PAD, peripheral arterial disease; PAH, pulmonary arterial hypertension.

Initial findings indicated that basal constitutive cardiac-specific autophagy impairment, obtained by a tamoxifen-induced deletion of *Atg5*, shows disorganized sarcomere structure, mitochondrial misalignment and aggregation in cardiac myocytes, and cardiac hypertrophy, left ventricular dilatation, and contractile dysfunction, indicating the occurrence of HFrEF.[145] HFrEF progression can be prevented by rapamycin.[227] In fact, activation of basal autophagy by rapamycin, improves diastolic function in old mice beginning at 2–4 weeks and progress throughout 10-week treatment. Autophagy was transiently induced during the first week of treatment associated with mitochondrial biogenesis, suggesting the replacement of damaged mitochondria.[228] In a rat model of HFrEF induced by MI, histological and echocardiographic measurements showed that rapamycin treatment improves myocardial function and inhibits cardiac remodelling at 8 weeks post-MI mechanism involving inhibition of the MTORC1 and ER stress pathways.[229] In the same model, the increase of MMP9 (matrix metallopeptidase 9) inhibits cardiomyocyte and cardiac fibroblast autophagy in the peri-infarct site, whereas ablation of MMP9 increases cardiac autophagy.[230] Moreover, in a model of HF induced by pressure overload, nitric oxide and natriuretic peptides induce cardioprotection by activation of autophagy through a PRKG1–TSC2–RHEB-dependent inhibition of MTORC1.[231] In patients with dilated cardiomyopathy, autophagic vacuoles in cardiomyocytes are associated with a better HFrEF prognosis, suggesting autophagy could be involved in the prevention of myocardial deterioration.[232] In patients with idiopathic dilated cardiomyopathy, mechanical unloading with a left ventricular assist device decreases markers of autophagy BECN1, ATG12–ATG5, and LC3-II.[146] Because the mechanical unloading decreases the energy demand of the failing heart these results suggest that autophagy is activated as an adaptive mechanism. Moreover, myocardial CTSD, a major lysosomal protease induced by MI, protects against cardiac dysfunction and remodelling, which is in part through promoting cardiac myocyte autophagic flux.[233]

Conversely, autophagy, assessed by anti-LC3-II staining and vacuole formation, is extensively detected in myocardial samples from patients with ischemic cardiomyopathy and idiopathic dilated cardiomyopathy, with the occurrence of necroptosis and oncosis exceeding that of apoptosis.[234] In a mouse model of MI, necroptosis is triggered by autophagy flux dysfunction, and this cell death process contributes to loss of cardiomyocytes, adverse ventricular remodelling, and HFrEF.[235] Moreover, persistent autophagy triggered by upregulation of TLR3 expression and signalling induced by MI, promotes HFrEF and lethality.[236] Likewise, in a model of pressure overload in mice induced by TAC, left ventricular wall thickness is increased at 1 week, associated with a decrease in autophagy.[237] At 9 weeks, this model develops HFrEF characterized by a further increase in left ventricular wall thickness, with the intensification of cardiomyocyte autophagy and apoptosis.[237] In this model, activation of AMPK by injecting AICAR improves cardiac function by attenuating autophagy.[238] These results suggest that in HFrEF, autophagy can be beneficial or detrimental, depending on the context of the disease.

14. Cardiac ageing and regeneration

Cardiac ageing is characterized by fibrosis, hypertrophy, dysfunctional mitochondria, and misfolded protein accumulation, and impaired autophagy.[239] Inhibition of autophagy exacerbates cardiac dysfunction accompanied by the accumulation of dysfunctional organelles and misfolded proteins.[240] Reduction of autophagy during ageing correlates with the hypermethylation of *Atg5* and *Lc3b* gene promoter regions in macrophages from aged mice associated with reduced gene expression;[241] treatment with *Trdmt1*/*Dnmt2* siRNA or a methyltransferase inhibitor restores *Atg5* and *Lc3* expression.[241] Interestingly, a homozygous $BECN1^{F121A}$ knock-in mouse, a mutation that decreases the interaction of BECN1 with the negative regulator BCL2, has increased basal autophagy in several organs, including the heart.[242] These mice show an increased life span with decreased cardiac and renal pathological changes and spontaneous tumorigenesis.[242]

Conversely, cardiac regeneration is considered a promising tool to treat cardiac diseases. Zebrafish, but not adult mammals, are capable of cardiac tissue regeneration. In an injury model of ventricular apex resection, a tightly regulated autophagic response is observed during the early stages of the regeneration process. Inhibition of autophagy by rapamycin impairs cardiac regeneration.[243] These findings clearly show the double-edged sword effect that autophagy has in cardiac homeostasis.

15. Autophagy in the vascular system

The vascular tissue is mainly constituted by vascular smooth muscle cells (VSMCs) and endothelial cells. VSMCs exhibits several phenotypes in response to endogenous and environmental factors contributing to the genesis and progression of vascular diseases.[244] Comprehensive overviews of the role of autophagy in VSMCs were previously and extensively described.[134,135]

16. VSMC phenotype

Several studies have shown that autophagy controls VSMC differentiation and dedifferentiation. Dedifferentiated VSMCs are characterized by increased cell migration, proliferation, extracellular matrix protein synthesis, secretion, and decreased contractile protein content.[244] This phenotype is responsible for vessel formation and reparation. However, when it is dysregulated, it is responsible for vascular diseases (*Figure 5*).[244] Inhibition of autophagy with 3-methyladenine delays the differentiation of epicardial progenitor cells into coronary VSMCs while its activation with rapamycin induces an early transient differentiation of epicardial progenitor cells and enhanced migration.[245] Moreover, GDF11, an inhibitor of cardiac hypertrophy in mice,[246] promotes carotid VSMC differentiation and prevents cell dedifferentiation induced by autophagosome accumulation triggered by western diet, lysosome function deficiency, and inflammation.[247]

Conversely, angiotensin II (Ang II), through an AGTR1–RHOA kinase-dependent signalling pathway, activates autophagy in VSMCs by a mechanism involving the increase of BECN1, PIK3C3, ATG12–ATG5, ATG4, and ATG7 protein levels and BECN1 phosphorylation, suggesting activation of phagophore initiation and expansion.[248] Ang II regulates contractile protein content in VSMCs through an autophagy-dependent mechanism.[248] Inhibition of autophagy by SEPTIN4,[249] and cortistatin, a neuropeptide highly homologous to somatostatin,[250] inhibits Ang II-induced VSMC proliferation. Similarly, an AMPK–MTORC1 signalling-dependent induction of autophagy in VSMCs is described for SPARC. SPARC upregulates LC3-II, BECN1, and ATG5, triggering an autophagy-dependent VSMC dedifferentiation.[251] Regulation of cell migration by autophagy via the degradation of KAT2A is also described for VSMC.[252] Therefore, VSMC dedifferentiation processes, i.e. proliferation, migration, contractile, and extracellular matrix protein synthesis, are highly regulated by autophagy.

17. Endothelial function

The endothelium is a critical regulator of vascular health and function. Shear stress generated by the blood flow is one of the most important stimuli that control endothelial function.[253] Steady laminar shear stress (5 or 15 dynes/cm^2) induces autophagy in human and rabbit endothelial cells, associated with the induction of endothelial NOS3/eNOS (nitric oxide synthase 3) and inhibition of expression of the vasoconstrictor EDN1/ET-1 (endothelin 1).[156] The mechanism involves the deacetylation of ATG5 and ATG7 in a SIRT1-dependent manner.[254] In contrast, oscillatory or low levels of shear stress reduce autophagy, uncouple NOS3, and trigger proatherogenic responses.[168,254,255] From a translational point of view, using primary endothelial cells collected from the radial artery of men, showed that 60 min of rhythmic handgrip exercise activates autophagy, increases NOS3 phosphorylation, and increases nitric oxide and $O_2^{\cdot-}$ generation.[256] In diabetic patients, autophagic flux is reduced, which impairs NOS3 activation.[163] These data suggest that steady laminar shear stress induces autophagy with a beneficial vasodilatory effect, and diabetes disrupts these mechanisms. Moreover, disturbed blood flow promotes atherosclerosis, whereas laminar flow has a protective action by preventing endothelial apoptosis, senescence, and inflammation.[169] The mechanism involves activation of endothelial autophagy that triggers SIRT1 expression that inhibits STK3/MST2–STK4/MST1 (HIPPO)-YAP1 signalling that interrupts atherosclerotic plaque formation.[188]

18. Vascular remodelling

Pathological vascular remodelling is characterized by the narrowing of the vessel lumen, mobilization of VSMC to the intima layer, exacerbated extracellular matrix production (fibrosis) and infiltration by immune cells.[257] Dedifferentiated VSMCs have been extensively described in the development and progression of atherosclerosis, hypertension, restenosis, and neointimal formation.[258,259] Various rapamycin analogues (rapalogs) have been used in drug-eluting stents for treating arterial restenosis. Two major rapalogs, everolimus, and biolimus strongly inhibit neointimal hyperplasia.[260] However, everolimus exerts cytotoxicity by increasing ROS and reducing energy metabolism. In contrast, biolimus preferentially induces autophagy by activating ULK1. The implantation of biolimus-eluting stent reduces endothelial loss and inflammation in porcine coronary arteries.[260]

VSMCs with specific genetic *atg7* deletion show a reduction in serum-induced cell growth and cell proliferation rate and an increase in cell death.[261] Furthermore, an *atg7* conditional knockout mouse crossed with *Apoe* (apolipoprotein E)-deficient mouse shows a reduction in medial cellularity and an increase in TUNEL-positive cells in the descending aorta. This mouse also shows a reduced survival rate due to aortic

rupture associated with increased markers of atherosclerosis.[261] Using another VSMC-specific *atg7* knockout mouse continuously infused with Ang II (1.5 mg/kg/day) also exhibits increased mortality associated with cardiac rupture, MI, end-organ damage, venous distention, pleural effusion, and abdominal aortic dissections.[262] Deletion of *Atg5* in VSMCs increases cell death susceptibility, enhances ER stress activation, promotes VSMC inflammation, and increases the incidence of fatal rupture of aortic aneurysm (*Figure 5*).[181] TFEB is downregulated in aortic aneurysm lesions, and TFEB deficiency increases VSMC apoptosis and promotes abdominal aortic aneurysm formation in mice.[263] In contrast, TFEB activation has the opposite effect.[263] Additionally, metformin preserves aortic elastin and collagen and reduces aortic cell apoptosis abdominal aortic aneurysms in patients and rat models. The mechanism involves the inactivation of the PI3K–AKT–MTORC1 pathway and the decrease of mRNA and protein levels of LC3B and BECN1.[264] Inhibition of vascular inflammation by using BP-1-102, a novel potent STAT3 inhibitor, decreases abdominal aortic aneurysm by preserving autophagy.[265] These data suggest that the fine-tuning of autophagy flux is required to maintain aorta wall integrity.

19. Atherosclerosis

VSMC dedifferentiation is a key step in the development of atherosclerotic plaque. Therefore, disruption of autophagy has important consequences on atherosclerosis genesis and progression. Besides macrophages, VSMCs are also an important source of foam cells in atherosclerosis. In a high-fat diet-fed *apoe* knockout mice model of atherosclerosis, activation of the P2RY12 receptor inhibits autophagy in VSMCs, which decreases cholesterol efflux and promotes VSMC-derived foam cell formation.[266]

Atherosclerotic plaque instability predisposes to the occurrence of cardiovascular events such as MI and stroke. A mouse model of plaque instability in VSMC-specific *atg7* knockout mice crossed with *apoe* knockout mice, shows that defective autophagy in VSMCs increases plaque instability and the risk of rupture (*Figure 5*).[267] Conversely, enhancement of autophagy in VSMCs or macrophages by using trehalose or by overexpression of TFEB exerts athero-protective effects, reducing plaque formation with a reduction of inflammation.[268] Moreover, in the context of proatherogenic stimuli, the deficiency of autophagy induces inflammation in coronary artery VSMCs by promoting NLRP3 inflammasome formation and activation.[269] These data suggest that autophagy is also a critical process in maintaining immune homeostasis during atherosclerosis.

20. Pulmonary hypertension

Pulmonary hypertension (PH) is a progressive disease characterized by excessive proliferation of pulmonary arterial vascular smooth muscle cells (PASMC).[270] More than 70% of familial PH and 20% of idiopathic PH patients carry heterozygous mutations in BMPR2.[271] BMPR2 is degraded by autophagy in pulmonary artery endothelial cells and PASMC.[271] Mutations in BMPR2 are sufficient to trigger an increased autophagic flux.[271] Similarly, pulmonary microvascular endothelial cells from end-stage idiopathic PH patients present an elevated autophagic flux.[271] Along these lines, inhibition of autophagy with chloroquine[272] or paclitaxel[273] blocks PASMC proliferation (*Figure 5*).

21. Autophagy as a pharmacological target in cardiovascular diseases and current limitations

Despite several pharmacological agents and regimens that modulate autophagy, very few clinical trials have evaluated their effects in cardiovascular diseases. Of these, only exercise, trehalose, and hydroxychloroquine have been evaluated (*Table 2*). Another important current limitation in the field is the lack of cell/organ-targeted delivery of exogenous autophagy regulators for cardiac or vascular repair. We are still far from having an effective autophagy therapy. However, insights obtained from such works will provide inspiration for future clinical trials to assess the autophagic response for therapeutic gain. Careful consideration must be kept in mind because of the dual role of autophagy in cytoprotection and cell death.

22. Conclusions

As autophagy remains an important factor in maintaining cardiovascular homeostasis, further research is required to pinpoint novel pharmacological targets that will allow us to harness the benefits of controlling autophagy levels to treat different cardiac pathologies.

Funding

This work was supported by grants from the National Institutes of Health (GM131919 to D.J.K.), the Agencia Nacional de Investigacion y Desarrollo (ANID), Chile: Fondo Nacional de Desarrollo Científico y Tecnológico/FONDECYT (1180157 to M.C. and 120049 to S.L.), and Fund for Financing Research Centers in Priority Areas/FONDAP (15130011 to M.C. and S.L.).

Conflict of interest: none declared.

References

1. Lahiri V, Hawkins WD, Klionsky DJ. Watch what you (self-) eat: autophagic mechanisms that modulate metabolism. *Cell Metab* 2019;**29**:803–826.
2. Mazure NM, Pouyssegur J. Hypoxia-induced autophagy: cell death or cell survival? *Curr Opin Cell Biol* 2010;**22**:177–180.
3. Smith MD, Harley ME, Kemp AJ, Wills J, Lee M, Arends M, von Kriegsheim A, Behrends C, Wilkinson S. CCPG1 is a non-canonical autophagy cargo receptor essential for ER-phagy and pancreatic ER proteostasis. *Dev Cell* 2018;**44**:217–232.e211.
4. Fumagalli F, Noack J, Bergmann TJ, Cebollero E, Pisoni GB, Fasana E, Fregno I, Galli C, Loi M, Solda T, D'Antuono R, Raimondi A, Jung M, Melnyk A, Schorr S, Schreiber A, Simonelli L, Varani L, Wilson-Zbinden C, Zerbe O, Hofmann K, Peter M, Quadroni M, Zimmermann R, Molinari M. Translocon component Sec62 acts in endoplasmic reticulum turnover during stress recovery. *Nat Cell Biol* 2016;**18**:1173–1184.
5. Cuervo AM, Wong E.Chaperone-mediated autophagy: roles in disease and aging. Cell Res 2014;**24**:92–104.
6. Schuck S.Microautophagy- distinct molecular mechanisms handle cargoes of many sizes. J Cell Sci 2020;**133**:jcs246322.doi: 10.1242/jcs.246322.
7. Pedrozo Z, Torrealba N, Fernandez C, Gatica D, Toro B, Quiroga C, Rodriguez AE, Sanchez G, Gillette TG, Hill JA, Donoso P, Lavandero S. Cardiomyocyte ryanodine receptor degradation by chaperone-mediated autophagy. *Cardiovasc Res* 2013;**98**:277–285.
8. Feng Y, Yao Z, Klionsky DJ. How to control self-digestion: transcriptional, post-transcriptional, and post-translational regulation of autophagy. *Trends Cell Biol* 2015;**25**:354–363.
9. Gatica D, Hu G, Liu X, Zhang N, Williamson PR, Klionsky DJ. The Pat1-Lsm complex stabilizes ATG mRNA during nitrogen starvation-induced autophagy. *Mol Cell* 2019;**73**:314–324.e314.
10. Ganley IG, Lam Du H, Wang J, Ding X, Chen S, Jiang X. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J Biol Chem* 2009;**284**:12297–12305.

11. Chan EY, Kir S, Tooze SA. siRNA screening of the kinome identifies ULK1 as a multidomain modulator of autophagy. *J Biol Chem* 2007;**282**:25464–25474.
12. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, Iemura S, Natsume T, Takehana K, Yamada N, Guan JL, Oshiro N, Mizushima N. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* 2009;**20**:1981–1991.
13. Chan EY, Longatti A, McKnight NC, Tooze SA. Kinase-inactivated ULK proteins inhibit autophagy via their conserved C-terminal domains using an Atg13-independent mechanism. *Mol Cell Biol* 2009;**29**:157–171.
14. Hara T, Takamura A, Kishi C, Iemura S, Natsume T, Guan JL, Mizushima N. FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. *J Cell Biol* 2008;**181**:497–510.
15. Hosokawa N, Sasaki T, Iemura S, Natsume T, Hara T, Mizushima N. Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy* 2009;**5**:973–979.
16. Bach M, Larance M, James DE, Ramm G. The serine/threonine kinase ULK1 is a target of multiple phosphorylation events. *Biochem J* 2011;**440**:283–291.
17. Dorsey FC, Rose KL, Coenen S, Prater SM, Cavett V, Cleveland JL, Caldwell-Busby J. Mapping the phosphorylation sites of Ulk1. *J Proteome Res* 2009;**8**:5253–5263.
18. Russell RC, Tian Y, Yuan H, Park HW, Chang YY, Kim J, Kim H, Neufeld TP, Dillin A, Guan KL. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol* 2013;**15**:741–750.
19. Egan DF, Chun MG, Vamos M, Zou H, Rong J, Miller CJ, Lou HJ, Raveendra-Panickar D, Yang CC, Sheffler DJ, Teriete P, Asara JM, Turk BE, Cosford ND, Shaw RJ. Small molecule inhibition of the autophagy kinase ULK1 and identification of ULK1 substrates. *Mol Cell* 2015;**59**:285–297.
20. Park JM, Jung CH, Seo M, Otto NM, Grunwald D, Kim KH, Moriarity B, Kim YM, Starker C, Nho RS, Voytas D, Kim DH. The ULK1 complex mediates MTORC1 signaling to the autophagy initiation machinery via binding and phosphorylating ATG14. *Autophagy* 2016;**12**:547–564.
21. Tian W, Alsaadi R, Guo Z, Kalinina A, Carrier M, Tremblay ME, Lacoste B, Lagace D, Russell RC. An antibody for analysis of autophagy induction. *Nat Methods* 2020; **17**:232–239.
22. Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, Kundu M, Kim DH. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* 2009;**20**:1992–2003.
23. Joo JH, Dorsey FC, Joshi A, Hennessy-Walters KM, Rose KL, McCastlain K, Zhang J, Iyengar R, Jung CH, Suen DF, Steeves MA, Yang CY, Prater SM, Kim DH, Thompson CB, Youle RJ, Ney PA, Cleveland JL, Kundu M. Hsp90-Cdc37 chaperone complex regulates Ulk1- and Atg13-mediated mitophagy. *Mol Cell* 2011;**43**:572–585.
24. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 2011;**13**:132–141.
25. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 2008;**320**:1496–1501.
26. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 2003;**17**:1829–1834.
27. Martina JA, Chen Y, Gucek M, Puertollano R. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy* 2012;**8**:903–914.
28. Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, Di Malta C, Donaudy F, Embrione V, Polishchuk RS, Banfi S, Parenti G, Cattaneo E, Ballabio A. A gene network regulating lysosomal biogenesis and function. *Science* 2009;**325**:473–477.
29. Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P, Sardiello M, Rubinsztein DC, Ballabio A. TFEB links autophagy to lysosomal biogenesis. *Science* 2011;**332**:1429–1433.
30. Degtyarev M, De Maziere A, Orr C, Lin J, Lee BB, Tien JY, Prior WW, van Dijk S, Wu H, Gray DC, Davis DP, Stern HM, Murray LJ, Hoeflich KP, Klumperman J, Friedman LS, Lin K. Akt inhibition promotes autophagy and sensitizes PTEN-null tumors to lysosomotropic agents. *J Cell Biol* 2008;**183**:101–116.
31. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* 2002;**10**:151–162.
32. Nave BT, Ouwens M, Withers DJ, Alessi DR, Shepherd PR. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem J* 1999;**344(Pt 2)**:427–431.
33. Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, Ogier-Denis E. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J Biol Chem* 2001;**276**: 35243–35246.
34. Wang RC, Wei Y, An Z, Zou Z, Xiao G, Bhagat G, White M, Reichelt J, Levine B. Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. *Science* 2012;**338**:956–959.
35. Sengupta A, Molkentin JD, Yutzey KE. FoxO transcription factors promote autophagy in cardiomyocytes. *J Biol Chem* 2009;**284**:28319–28331.
36. Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, Burden SJ, Di Lisi R, Sandri C, Zhao J, Goldberg AL, Schiaffino S, Sandri M. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab* 2007;**6**:458–471.
37. Zhao J, Brault JJ, Schild A, Cao P, Sandri M, Schiaffino S, Lecker SH, Goldberg AL. FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. *Cell Metab* 2007;**6**:472–483.
38. Scott JW, Hawley SA, Green KA, Anis M, Stewart G, Scullion GA, Norman DG, Hardie DG. CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J Clin Invest* 2004;**113**:274–284.
39. Cheung PC, Salt IP, Davies SP, Hardie DG, Carling D. Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem J* 2000;**346(Pt 3)**:659–669.
40. Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Makela TP, Alessi DR, Hardie DG. Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* 2003;**2**:28.
41. Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LG, Neumann D, Schlattner U, Wallimann T, Carlson M, Carling D. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol* 2003;**13**:2004–2008.
42. Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, Cantley LC. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci USA* 2004;**101**: 3329–3335.
43. Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, Asara JM, Fitzpatrick J, Dillin A, Viollet B, Kundu M, Hansen M, Shaw RJ. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 2011;**331**:456–461.
44. Kim J, Kim YC, Fang C, Russell RC, Kim JH, Fan W, Liu R, Zhong Q, Guan KL. Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell* 2013;**152**:290–303.
45. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003;**115**:577–590.
46. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* 2008;**30**:214–226.
47. Loffler AS, Alers S, Dieterle AM, Keppeler H, Franz-Wachtel M, Kundu M, Campbell DG, Wesselborg S, Alessi DR, Stork B. Ulk1-mediated phosphorylation of AMPK constitutes a negative regulatory feedback loop. *Autophagy* 2011;**7**: 696–706.
48. Holczer M, Hajdú B, Lőrincz T, Szarka A, Bánhegyi G, Kapuy O. Fine-tuning of AMPK-ULK1-mTORC1 regulatory triangle is crucial for autophagy oscillation. *Sci Rep* 2020;**10**:17803.
49. Dohmen M, Krieg S, Agalaridis G, Zhu X, Shehata SN, Pfeiffenberger E, Amelang J, Butepage M, Buerova E, Pfaff CM, Chanda D, Geley S, Preisinger C, Sakamoto K, Luscher B, Neumann D, Vervoorts J. AMPK-dependent activation of the Cyclin Y/CDK16 complex controls autophagy. *Nat Commun* 2020;**11**:1032.
50. Cherra SJ 3rd, Kulich SM, Uechi G, Balasubramani M, Mountzouris J, Day BW, Chu CT. Regulation of the autophagy protein LC3 by phosphorylation. *J Cell Biol* 2010; **190**:533–539.
51. Zhao X, Nedvetsky P, Stanchi F, Vion AC, Popp O, Zuhlke K, Dittmar G, Klussmann E, Gerhardt H. Endothelial PKA activity regulates angiogenesis by limiting autophagy through phosphorylation of ATG16L1. *Elife* 2019;**8**:e46380.
52. Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouysségur J, Mazure NM. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol* 2009;**29**:2570–2581.
53. Tracy K, Dibling BC, Spike BT, Knabb JR, Schumacker P, Macleod KF. BNIP3 is an RB/E2F target gene required for hypoxia-induced autophagy. *Mol Cell Biol* 2007;**27**: 6229–6242.
54. Park JM, Seo M, Jung CH, Grunwald D, Stone M, Otto NM, Toso E, Ahn Y, Kyba M, Griffin TJ, Higgins L, Kim DH. ULK1 phosphorylates Ser30 of BECN1 in association with ATG14 to stimulate autophagy induction. *Autophagy* 2018;**14**:584–597.
55. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, Murakami T, Taniguchi M, Tanii I, Yoshinaga K, Shiosaka S, Hammarback JA, Urano F, Imaizumi K. Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* 2006;**26**:9220–9231.
56. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 2000;**287**:664–666.
57. Wei Y, Pattingre S, Sinha S, Bassik M, Levine B. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol Cell* 2008;**30**:678–688.
58. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, Ron D. Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* 2000;**6**:1099–1108.
59. Kouroku Y, Fujita E, Tanida I, Ueno T, Isoai A, Kumagai H, Ogawa S, Kaufman RJ, Kominami E, Momoi T. ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation. *Cell Death Differ* 2007;**14**:230–239.
60. Wang J, Li Y, Duan J, Yang M, Yu Y, Feng L, Yang X, Zhou X, Zhao Z, Sun Z. Silica nanoparticles induce autophagosome accumulation via activation of the EIF2AK3 and ATF6 UPR pathways in hepatocytes. *Autophagy* 2018;**14**:1185–1200.

61. B'Chir W, Maurin AC, Carraro V, Averous J, Jousse C, Muranishi Y, Parry L, Stepien G, Fafournoux P, Bruhat A. The eIF2alpha/ATF4 pathway is essential for stress-induced autophagy gene expression. *Nucleic Acids Res* 2013;**41**:7683–7699.
62. Rzymski T, Milani M, Pike L, Buffa F, Mellor HR, Winchester L, Pires I, Hammond E, Ragoussis I, Harris AL. Regulation of autophagy by ATF4 in response to severe hypoxia. *Oncogene* 2010;**29**:4424–4435.
63. Ranjitha HB, Ammanathan V, Guleria N, Hosamani M, Sreenivasa BP, Dhanesh VV, Santhoshkumar R, Sagar BKC, Mishra BP, Singh RK, Sanyal A, Manjithaya R, Basagoudanavar SH. Foot-and-mouth disease virus induces PERK-mediated autophagy to suppress the antiviral interferon response. *J Cell Sci* 2020;**134**:jcs240622.
64. Luhr M, Torgersen ML, Szalai P, Hashim A, Brech A, Staerk J, Engedal N. The kinase PERK and the transcription factor ATF4 play distinct and essential roles in autophagy resulting from tunicamycin-induced ER stress. *J Biol Chem* 2019;**294**:8197–8217.
65. Grumati P, Dikic I, Stolz A. ER-phagy at a glance. *J Cell Sci* 2018;**131**:jcs217364.
66. Wilkinson S. ER-phagy: shaping up and destressing the endoplasmic reticulum. *FEBS J* 2019;**286**:2645–2663.
67. Lin DS, Huang YW, Ho CS, Hung PL, Hsu MH, Wang TJ, Wu TY, Lee TH, Huang ZD, Chang PC, Chiang MF. Oxidative insults and mitochondrial DNA mutation promote enhanced autophagy and mitophagy compromising cell viability in pluripotent cell model of mitochondrial disease. *Cells* 2019;**8**:65.
68. Chen Y, Azad MB, Gibson SB. Superoxide is the major reactive oxygen species regulating autophagy. *Cell Death Differ* 2009;**16**:1040–1052.
69. Chen L, Xu B, Liu L, Luo Y, Yin J, Zhou H, Chen W, Shen T, Han X, Huang S. Hydrogen peroxide inhibits mTOR signaling by activation of AMPKalpha leading to apoptosis of neuronal cells. *Lab Invest* 2010;**90**:762–773.
70. Polson HE, de Lartigue J, Rigden DJ, Reedijk M, Urbe S, Clague MJ, Tooze SA. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. *Autophagy* 2010;**6**:506–522.
71. Kihara A, Kabeya Y, Ohsumi Y, Yoshimori T. Beclin-phosphatidylinositol 3-kinase complex functions at the trans-Golgi network. *EMBO Rep* 2001;**2**:330–335.
72. 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**:5360–5372.
73. Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, Maejima I, Shirahama-Noda K, Ichimura T, Isobe T, Akira S, Noda T, Yoshimori T. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat Cell Biol* 2009;**11**:385–396.
74. 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 USA* 2008;**105**:19211–19216.
75. Lu J, He L, Behrends C, Araki M, Araki K, Jun Wang Q, Catanzaro JM, Friedman SL, Zong WX, Fiel MI, Li M, Yue Z. NRBF2 regulates autophagy and prevents liver injury by modulating Atg14L-linked phosphatidylinositol-3 kinase III activity. *Nat Commun* 2014;**5**:3920.
76. Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, Corazzari M, Fuoco C, Ucar A, Schwartz P, Gruss P, Piacentini M, Chowdhury K, Cecconi F. Ambra1 regulates autophagy and development of the nervous system. *Nature* 2007;**447**:1121–1125.
77. Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, Jung JU. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* 2006;**8**:688–699.
78. Takahashi Y, Coppola D, Matsushita N, Cualing HD, Sun M, Sato Y, Liang C, Jung JU, Cheng JQ, Mule JJ, Pledger WJ, Wang HG. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* 2007;**9**:1142–1151.
79. Liang C, Lee JS, Inn KS, Gack MU, Li Q, Roberts EA, Vergne I, Deretic V, Feng P, Akazawa C, Jung JU. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat Cell Biol* 2008;**10**:776–787.
80. Kim YM, Jung CH, Seo M, Kim EK, Park JM, Bae SS, Kim DH. mTORC1 phosphorylates UVRAG to negatively regulate autophagosome and endosome maturation. *Mol Cell* 2015;**57**:207–218.
81. Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A. A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat Cell Biol* 2009;**11**:1433–1437.
82. Young AR, Chan EY, Hu XW, Kochl R, Crawshaw SG, High S, Hailey DW, Lippincott-Schwartz J, Tooze SA. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J Cell Sci* 2006;**119**:3888–3900.
83. Hailey DW, Rambold AS, Satpute-Krishnan P, Mitra K, Sougrat R, Kim PK, Lippincott-Schwartz J. Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* 2010;**141**:656–667.
84. Puri C, Renna M, Bento CF, Moreau K, Rubinsztein DC. Diverse autophagosome membrane sources coalesce in recycling endosomes. *Cell* 2013;**154**:1285–1299.
85. Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC. Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat Cell Biol* 2010;**12**:747–757.
86. Zhou C, Ma K, Gao R, Mu C, Chen L, Liu Q, Luo Q, Feng D, Zhu Y, Chen Q. Regulation of mATG9 trafficking by Src- and ULK1-mediated phosphorylation in basal and starvation-induced autophagy. *Cell Res* 2017;**27**:184–201.
87. Imai K, Hao F, Fujita N, Tsuji Y, Oe Y, Araki Y, Hamasaki M, Noda T, Yoshimori T. Atg9A trafficking through the recycling endosomes is required for autophagosome formation. *J Cell Sci* 2016;**129**:3781–3791.
88. Mattera R, Park SY, De Pace R, Guardia CM, Bonifacino JS. AP-4 mediates export of ATG9A from the trans-Golgi network to promote autophagosome formation. *Proc Natl Acad Sci USA* 2017;**114**:E10697–E10706.
89. Guardia CM, Tan XF, Lian T, Rana MS, Zhou W, Christenson ET, Lowry AJ, Faraldo-Gomez JD, Bonifacino JS, Jiang J, Banerjee A. Structure of human ATG9A, the only transmembrane protein of the core autophagy machinery. *Cell Rep* 2020;**31**:107837.
90. Maeda S, Yamamoto H, Kinch LN, Garza CM, Takahashi S, Otomo C, Grishin NV, Forli S, Mizushima N, Otomo T. Structure, lipid scrambling activity and role in autophagosome formation of ATG9A. *Nat Struct Mol Biol* 2020;**27**:1194–1201.
91. Maeda S, Otomo C, Otomo T. The autophagic membrane tether ATG2A transfers lipids between membranes. *Elife* 2019;**8**:e45777.
92. Valverde DP, Yu S, Boggavarapu V, Kumar N, Lees JA, Walz T, Reinisch KM, Melia TJ. ATG2 transports lipids to promote autophagosome biogenesis. *J Cell Biol* 2019;**218**:1787–1798.
93. Ge L, Melville D, Zhang M, Schekman R. The ER-Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis. *Elife* 2013;**2**:e00947.
94. Ge L, Zhang M, Schekman R. Phosphatidylinositol 3-kinase and COPII generate LC3 lipidation vesicles from the ER-Golgi intermediate compartment. *Elife* 2014;**3**:e04135.
95. Jeong YT, Simoneschi D, Keegan S, Melville D, Adler NS, Saraf A, Florens L, Washburn MP, Cavasotto CN, Fenyo D, Cuervo AM, Rossi M, Pagano M. The ULK1-FBXW5-SEC23B nexus controls autophagy. *Elife* 2018;**7**:e42253.
96. Yla-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL. 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* 2009;**5**:1180–1185.
97. Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A. Electron tomography reveals the endoplasmic reticulum as a membrane source for autophagosome formation. *Autophagy* 2010;**6**:301–303.
98. Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, Griffiths G, Ktistakis NT. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol* 2008;**182**:685–701.
99. Matsunaga K, Morita E, Saitoh T, Akira S, Ktistakis NT, Izumi T, Noda T, Yoshimori T. Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. *J Cell Biol* 2010;**190**:511–521.
100. 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**:238–252.
101. Otomo T, Chowdhury S, Lander GC. The rod-shaped ATG2A-WIPI4 complex tethers membranes in vitro. *Contact (Thousand Oaks)* 2018;**1**:251525641881993.
102. Chowdhury S, Otomo C, Leitner A, Ohashi K, Aebersold R, Lander GC, Otomo T. Insights into autophagosome biogenesis from structural and biochemical analyses of the ATG2A-WIPI4 complex. *Proc Natl Acad Sci USA* 2018;**115**:E9792–E9801.
103. Karanasios E, Stapleton E, Manifava M, Kaizuka T, Mizushima N, Walker SA, Ktistakis NT. Dynamic association of the ULK1 complex with omegasomes during autophagy induction. *J Cell Sci* 2013;**126**:5224–5238.
104. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, Oomori H, Noda T, Haraguchi T, Hiraoka Y, Amano A, Yoshimori T. Autophagosomes form at ER-mitochondria contact sites. *Nature* 2013;**495**:389–393.
105. Tang Z, Takahashi Y, He H, Hattori T, Chen C, Liang X, Chen H, Young MM, Wang HG. TOM40 targets Atg2 to mitochondria-associated ER membranes for phagophore expansion. *Cell Rep* 2019;**28**:1744–1757.e1745.
106. Biazik J, Yla-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL. Ultrastructural relationship of the phagophore with surrounding organelles. *Autophagy* 2015;**11**:439–451.
107. Mizushima N, Sugita H, Yoshimori T, Ohsumi Y. A new protein conjugation system in human. The counterpart of the yeast Apg12p conjugation system essential for autophagy. *J Biol Chem* 1998;**273**:33889–33892.
108. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, Yoshimori T. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* 2000;**19**:5720–5728.
109. Noda NN, Kumeta H, Nakatogawa H, Satoo K, Adachi W, Ishii J, Fujioka Y, Ohsumi Y, Inagaki F. Structural basis of target recognition by Atg8/LC3 during selective autophagy. *Genes Cells* 2008;**13**:1211–1218.
110. Nishimura T, Kaizuka T, Cadwell K, Sahani MH, Saitoh T, Akira S, Virgin HW, Mizushima N. FIP200 regulates targeting of Atg16L1 to the isolation membrane. *EMBO Rep* 2013;**14**:284–291.
111. Lystad AH, Carlsson SR, de la Ballina LR, Kauffman KJ, Nag S, Yoshimori T, Melia TJ, Simonsen A. Distinct functions of ATG16L1 isoforms in membrane binding and LC3B lipidation in autophagy-related processes. *Nat Cell Biol* 2019;**21**:372–383.
112. Gatica D, Lahiri V, Klionsky DJ. Cargo recognition and degradation by selective autophagy. *Nat Cell Biol* 2018;**20**:233–242.
113. Thurston TL, Ryzhakov G, Bloor S, von Muhlinen N, Randow F. The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. *Nat Immunol* 2009;**10**:1215–1221.

114. Klionsky DJ, Schulman BA. Dynamic regulation of macroautophagy by distinctive ubiquitin-like proteins. *Nat Struct Mol Biol* 2014;**21**:336–345.
115. Rogov VV, Stolz A, Ravichandran AC, Rios-Szwed DO, Suzuki H, Kniss A, Lohr F, Wakatsuki S, Dotsch V, Dikic I, Dobson RC, McEwan DG. Structural and functional analysis of the GABARAP interaction motif (GIM). *EMBO Rep* 2017;**18**:1382–1396.
116. Alemu EA, Lamark T, Torgersen KM, Birgisdottir AB, Larsen KB, Jain A, Olsvik H, Overvatn A, Kirkin V, Johansen T. 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**:39275–39290.
117. Birgisdottir AB, Mouilleron S, Bhujabal Z, Wirth M, Sjottem E, Evjen G, Zhang W, Lee R, O'Reilly N, Tooze SA, Lamark T, Johansen T. Members of the autophagy class III phosphatidylinositol 3-kinase complex I interact with GABARAP and GABARAPL1 via LIR motifs. *Autophagy* 2019;**15**:1333–1355.
118. Skytte Rasmussen M, Mouilleron S, Kumar Shrestha B, Wirth M, Lee R, Bowitz Larsen K, Abudu Princely Y, O'Reilly N, Sjøttem E, Tooze SA, Lamark T, Johansen T. ATG4B contains a C-terminal LIR motif important for binding and efficient cleavage of mammalian orthologs of yeast Atg8. *Autophagy* 2017;**13**:834–853.
119. Noda NN, Satoo K, Fujioka Y, Kumeta H, Ogura K, Nakatogawa H, Ohsumi Y, Inagaki F. Structural basis of Atg8 activation by a homodimeric E1, Atg7. *Mol Cell* 2011;**44**:462–475.
120. Marshall RS, Hua Z, Mali S, McLoughlin F, Vierstra RD. ATG8-binding UIM proteins define a new class of autophagy adaptors and receptors. *Cell* 2019;**177**:766–781.e724.
121. Kimura S, Noda T, Yoshimori T. Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes. *Cell Struct Funct* 2008;**33**:109–122.
122. Fass E, Shvets E, Degani I, Hirschberg K, Elazar Z. Microtubules support production of starvation-induced autophagosomes but not their targeting and fusion with lysosomes. *J Biol Chem* 2006;**281**:36303–36316.
123. Gutierrez MG, Munafo DB, Beron W, Colombo MI. Rab7 is required for the normal progression of the autophagic pathway in mammalian cells. *J Cell Sci* 2004;**117**:2687–2697.
124. Jager S, Bucci C, Tanida I, Ueno T, Kominami E, Saftig P, Eskelinen EL. Role for Rab7 in maturation of late autophagic vacuoles. *J Cell Sci* 2004;**117**:4837–4848.
125. Tian X, Zheng P, Zhou C, Wang X, Ma H, Ma W, Zhou X, Teng J, Chen J. DIPK2A promotes STX17- and VAMP7-mediated autophagosome-lysosome fusion by binding to VAMP7B. *Autophagy* 2020;**16**:797–810.
126. Saleeb RS, Kavanagh DM, Dun AR, Dalgarno PA, Duncan RR. A VPS33A-binding motif on syntaxin 17 controls autophagy completion in mammalian cells. *J Biol Chem* 2019;**294**:4188–4201.
127. Jiang P, Nishimura T, Sakamaki Y, Itakura E, Hatta T, Natsume T, Mizushima N. The HOPS complex mediates autophagosome-lysosome fusion through interaction with syntaxin 17. *Mol Biol Cell* 2014;**25**:1327–1337.
128. McEwan DG, Popovic D, Gubas A, Terawaki S, Suzuki H, Stadel D, Coxon FP, Miranda de Stegmann D, Bhogaraju S, Maddi K, Kirchof A, Gatti E, Helfrich MH, Wakatsuki S, Behrends C, Pierre P, Dikic I. PLEKHM1 regulates autophagosome-lysosome fusion through HOPS complex and LC3/GABARAP proteins. *Mol Cell* 2015;**57**:39–54.
129. Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones TD, Chantranupong L, Comb W, Wang T, Bar-Peled L, Zoncu R, Straub C, Kim C, Park J, Sabatini BL, Sabatini DM. Metabolism. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. *Science* 2015;**347**:188–194.
130. Rebsamen M, Pochini L, Stasyk T, de Araujo ME, Galluccio M, Kandasamy RK, Snijder B, Fauster A, Rudashevskaya EL, Bruckner M, Scorzoni S, Filipek PA, Huber KV, Bigenzahn JW, Heinz LX, Kraft C, Bennett KL, Indiveri C, Huber LA, Superti-Furga G. SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. *Nature* 2015;**519**:477–481.
131. Jung J, Genau HM, Behrends C. Amino acid-dependent mTORC1 regulation by the lysosomal membrane protein SLC38A9. *Mol Cell Biol* 2015;**35**:2479–2494.
132. Wyant GA, Abu-Remaileh M, Wolfson RL, Chen WW, Freinkman E, Danai LV, Vander Heiden MG, Sabatini DM. mTORC1 activator SLC38A9 is required to efflux essential amino acids from lysosomes and use protein as a nutrient. *Cell* 2017;**171**:642–654.e612.
133. Scalise M, Galluccio M, Pochini L, Cosco J, Trotta M, Rebsamen M, Superti-Furga G, Indiveri C. Insights into the transport side of the human SLC38A9 transceptor. *Biochim Biophys Acta Biomembr* 2019;**1861**:1558–1567.
134. Gatica D, Chiong M, Lavandero S, Klionsky DJ. Molecular mechanisms of autophagy in the cardiovascular system. *Circ Res* 2015;**116**:456–467.
135. Lavandero S, Chiong M, Rothermel BA, Hill JA. Autophagy in cardiovascular biology. *J Clin Invest* 2015;**125**:55–64.
136. Lavandero S, Troncoso R, Rothermel BA, Martinet W, Sadoshima J, Hill JA. Cardiovascular autophagy: concepts, controversies, and perspectives. *Autophagy* 2013;**9**:1455–1466.
137. Morales PE, Arias-Duran C, Avalos-Guajardo Y, Aedo G, Verdejo HE, Parra V, Lavandero S. Emerging role of mitophagy in cardiovascular physiology and pathology. *Mol Aspects Med* 2020;**71**:100822.
138. Zhang J, Liu J, Huang Y, Chang JY, Liu L, McKeehan WL, Martin JF, Wang F. FRS2alpha-mediated FGF signals suppress premature differentiation of cardiac stem cells through regulating autophagy activity. *Circ Res* 2012;**110**:e29–e39.
139. Lee E, Koo Y, Ng A, Wei Y, Luby-Phelps K, Juraszek A, Xavier RJ, Cleaver O, Levine B, Amatruda JF. Autophagy is essential for cardiac morphogenesis during vertebrate development. *Autophagy* 2014;**10**:572–587.
140. Sybers HD, Ingwall J, DeLuca M. Autophagy in cardiac myocytes. *Recent Adv Stud Cardiac Struct Metab* 1976;**12**:453–463.
141. Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lullmann-Rauch R, Janssen PM, Blanz J, von Figura K, Saftig P. Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* 2000;**406**:902–906.
142. Shimomura H, Terasaki F, Hayashi T, Kitaura Y, Isomura T, Suma H. Autophagic degeneration as a possible mechanism of myocardial cell death in dilated cardiomyopathy. *Jpn Circ J* 2001;**65**:965–968.
143. Kostin S, Pool L, ElsäSser A, Hein S, Drexler HCA, Arnon E, Hayakawa Y, Zimmermann R, Bauer E, KlöVekorn W-P, Schaper J, Myocytes die by multiple mechanisms in failing human hearts. *Circ Res* 2003;**92**:715–724.
144. Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, Sadoshima J. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. *Circ Res* 2007;**100**:914–922.
145. Nakai A, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M, Omiya S, Mizote I, Matsumura Y, Asahi M, Nishida K, Hori M, Mizushima N, Otsu K. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. *Nat Med* 2007;**13**:619–624.
146. Kassiotis C, Ballal K, Wellnitz K, Vela D, Gong M, Salazar R, Frazier OH, Taegtmeyer H. Markers of autophagy are downregulated in failing human heart after mechanical unloading. *Circulation* 2009;**120**:S191–S197.
147. Hariharan N, Maejima Y, Nakae J, Paik J, Depinho RA, Sadoshima J. Deacetylation of FoxO by Sirt1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. *Circ Res* 2010;**107**:1470–1482.
148. Mai S, Muster B, Bereiter-Hahn J, Jendrach M. Autophagy proteins LC3B, ATG5 and ATG12 participate in quality control after mitochondrial damage and influence lifespan. *Autophagy* 2012;**8**:47–62.
149. Aránguiz-Urroz P, Canales J, Copaja M, Troncoso R, Vicencio JM, Carrillo C, Lara H, Lavandero S, Díaz-Araya G. Beta(2)-adrenergic receptor regulates cardiac fibroblast autophagy and collagen degradation. *Biochim Biophys Acta* 2011;**1812**:23–31.
150. Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, Oyabu J, Murakawa T, Nakayama H, Nishida K, Akira S, Yamamoto A, Komuro I, Otsu K. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature* 2012;**485**:251–255.
151. Ma X, Liu H, Foyil SR, Godar RJ, Weinheimer CJ, Hill JA, Diwan A. Impaired autophagosome clearance contributes to cardiomyocyte death in ischemia/reperfusion injury. *Circulation* 2012;**125**:3170–3181.
152. Cao DJ, Jiang N, Blagg A, Johnstone JL, Gondalia R, Oh M, Luo X, Yang KC, Shelton JM, Rothermel BA, Gillette TG, Dorn GW, Hill JA. Mechanical unloading activates FoxO3 to trigger Bnip3-dependent cardiomyocyte atrophy. *J Am Heart Assoc* 2013;**2**:e000016.
153. Chen Y, Dorn GW 2nd. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science* 2013;**340**:471–475.
154. Torisu T, Torisu K, Lee IH, Liu J, Malide D, Combs CA, Wu XS, Rovira II, Fergusson MM, Weigert R, Connelly PS, Daniels MP, Komatsu M, Cao L, Finkel T. Autophagy regulates endothelial cell processing, maturation and secretion of von Willebrand factor. *Nat Med* 2013;**19**:1281–1287.
155. Maejima Y, Kyoi S, Zhai P, Liu T, Li H, Ivessa A, Sciarretta S, Del Re DP, Zablocki DK, Hsu CP, Lim DS, Isobe M, Sadoshima J. Mst1 inhibits autophagy by promoting the interaction between Beclin1 and Bcl-2. *Nat Med* 2013;**19**:1478–1488.
156. Guo F, Li X, Peng J, Tang Y, Yang Q, Liu L, Wang Z, Jiang Z, Xiao M, Ni C, Chen R, Wei D, Wang GX. Autophagy regulates vascular endothelial cell eNOS and ET-1 expression induced by laminar shear stress in an ex vivo perfused system. *Ann Biomed Eng* 2014;**42**:1978–1988.
157. Gong G, Song M, Csordas G, Kelly DP, Matkovich SJ, Dorn GW 2nd. Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice. *Science* 2015;**350**:aad2459.
158. Kageyama Y, Hoshijima M, Seo K, Bedja D, Sysa-Shah P, Andrabi SA, Chen W, Hoke A, Dawson VL, Dawson TM, Gabrielson K, Kass DA, Iijima M, Sesaki H. Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain. *EMBO J* 2014;**33**:2798–2813.
159. Ikeda Y, Shirakabe A, Maejima Y, Zhai P, Sciarretta S, Toli J, Nomura M, Mihara K, Egashira K, Ohishi M, Abdellatif M, Sadoshima J. Endogenous Drp1 mediates mitochondrial autophagy and protects the heart against energy stress. *Circ Res* 2015;**116**:264–278.
160. Song M, Gong G, Burelle Y, Gustafsson AB, Kitsis RN, Matkovich SJ, Dorn GW 2nd. Interdependence of Parkin-mediated mitophagy and mitochondrial fission in adult mouse hearts. *Circ Res* 2015;**117**:346–351.
161. Araki S, Izumiya Y, Rokutanda T, Ianni A, Hanatani S, Kimura Y, Onoue Y, Senokuchi T, Yoshizawa T, Yasuda O, Koitabashi N, Kurabayashi M, Braun T, Bober E, Yamagata K, Ogawa H. Sirt7 contributes to myocardial tissue repair by maintaining transforming growth factor-beta signaling pathway. *Circulation* 2015;**132**:1081–1093.
162. Shirakabe A, Zhai P, Ikeda Y, Saito T, Maejima Y, Hsu CP, Nomura M, Egashira K, Levine B, Sadoshima J. Drp1-dependent mitochondrial autophagy plays a protective

role against pressure overload-induced mitochondrial dysfunction and heart failure. *Circulation* 2016;**133**:1249–1263.
163. Fetterman JL, Holbrook M, Flint N, Feng B, Breton-Romero R, Linder EA, Berk BD, Duess MA, Farb MG, Gokce N, Shirihai OS, Hamburg NM, Vita JA. Restoration of autophagy in endothelial cells from patients with diabetes mellitus improves nitric oxide signaling. *Atherosclerosis* 2016;**247**:207–217.
164. Li DL, Wang ZV, Ding G, Tan W, Luo X, Criollo A, Xie M, Jiang N, May H, Kyrychenko V, Schneider JW, Gillette TG, Hill JA. Doxorubicin blocks cardiomyocyte autophagic flux by inhibiting lysosome acidification. *Circulation* 2016;**133**:1668–1687.
165. Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, Harger A, Schipke J, Zimmermann A, Schmidt A, Tong M, Ruckenstuhl C, Dammbrueck C, Gross AS, Herbst V, Magnes C, Trausinger G, Narath S, Meinitzer A, Hu Z, Kirsch A, Eller K, Carmona-Gutierrez D, Büttner S, Pietrocola F, Knittelfelder O, Schrepfer E, Rockenfeller P, Simonini C, Rahn A, Horsch M, Moreth K, Beckers J, Fuchs H, Gailus-Durner V, Neff F, Janik D, Rathkolb B, Rozman J, de Angelis MH, Moustafa T, Haemmerle G, Mayr M, Willeit P, von Frieling-Salewsky M, Pieske B, Scorrano L, Pieber T, Pechlaner R, Willeit J, Sigrist SJ, Linke WA, Mühlfeld C, Sadoshima J, Dengjel J, Kiechl S, Kroemer G, Sedej S, Madeo F. Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat Med* 2016;**22**:1428–1438.
166. Yau JW, Singh KK, Hou Y, Lei X, Ramadan A, Quan A, Teoh H, Kuebler WM, Al-Omran M, Yanagawa B, Ni H, Verma S. Endothelial-specific deletion of autophagy-related 7 (ATG7) attenuates arterial thrombosis in mice. *J Thorac Cardiovasc Surg* 2017;**154**:978–988.e971.
167. Bharath LP, Cho JM, Park SK, Ruan T, Li Y, Mueller R, Bean T, Reese V, Richardson RS, Cai J, Sargsyan A, Pires K, Anandh Babu PV, Boudina S, Graham TE, Symons JD. Endothelial cell autophagy maintains shear stress-induced nitric oxide generation via glycolysis-dependent purinergic signaling to endothelial nitric oxide synthase. *Arterioscler Thromb Vasc Biol* 2017;**37**:1646–1656.
168. Vion AC, Kheloufi M, Hammoutene A, Poisson J, Lasselin J, Devue C, Pic I, Dupont N, Busse J, Stark K, Lafaurie-Janvore J, Barakat AI, Loyer X, Souyri M, Viollet B, Julia P, Tedgui A, Codogno P, Boulanger CM, Rautou PE. Autophagy is required for endothelial cell alignment and atheroprotection under physiological blood flow. *Proc Natl Acad Sci USA* 2017;**114**:E8675–E8684.
169. Kheloufi M, Vion AC, Hammoutene A, Poisson J, Lasselin J, Devue C, Pic I, Dupont N, Busse J, Stark K, Lafaurie-Janvore J, Barakat AI, Loyer X, Souyri M, Viollet B, Julia P, Tedgui A, Codogno P, Boulanger CM, Rautou PE. Endothelial autophagic flux hampers atherosclerotic lesion development. *Autophagy* 2018;**14**:173–175.
170. Sun Y, Yao X, Zhang QJ, Zhu M, Liu ZP, Ci B, Xie Y, Carlson D, Rothermel BA, Sun Y, Levine B, Hill JA, Wolf SE, Minei JP, Zang QS. Beclin-1-dependent autophagy protects the heart during sepsis. *Circulation* 2018;**138**:2247–2262.
171. Yang R, Song Z, Wu S, Wei Z, Xu Y, Shen X. Toll-like receptor 4 contributes to a myofibroblast phenotype in cardiac fibroblasts and is associated with autophagy after myocardial infarction in a mouse model. *Atherosclerosis* 2018;**279**:23–31.
172. Tong M, Saito T, Zhai P, Oka SI, Mizushima W, Nakamura M, Ikeda S, Shirakabe A, Sadoshima J. Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. *Circ Res* 2019;**124**:1360–1371.
173. Wang L, Ma H, Huang P, Xie Y, Near D, Wang H, Xu J, Yang Y, Xu Y, Garbutt T, Zhou Y, Liu Z, Yin C, Bressan M, Taylor JM, Liu J, Qian L. Down-regulation of Beclin1 promotes direct cardiac reprogramming. *Sci Transl Med* 2020;**12**:eaay7856.
174. Wildenthal K, Dees JH, Buja LM. Cardiac lysosomal derangements in mouse heart after long-term exposure to nonmetabolizable sugars. *Circ Res* 1977;**40**:26–35.
175. Dorn BR, Dunn WA Jr, Progulske-Fox A. Porphyromonas gingivalis traffics to autophagosomes in human coronary artery endothelial cells. *Infect Immun* 2001;**69**:5698–5708.
176. Jia G, Cheng G, Gangahar DM, Agrawal DK. Insulin-like growth factor-1 and TNF-alpha regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. *Immunol Cell Biol* 2006;**84**:448–454.
177. Lee SJ, Kim HP, Jin Y, Choi AM, Ryter SW. Beclin 1 deficiency is associated with increased hypoxia-induced angiogenesis. *Autophagy* 2011;**7**:829–839.
178. Grootaert MO, da Costa Martins PA, Bitsch N, Pintelon I, De Meyer GR, Martinet W, Schrijvers DM. Defective autophagy in vascular smooth muscle cells accelerates senescence and promotes neointima formation and atherogenesis. *Autophagy* 2015;**11**:2014–2032.
179. Docherty CK, Carswell A, Friel E, Mercer JR. Impaired mitochondrial respiration in human carotid plaque atherosclerosis: a potential role for Pink1 in vascular smooth muscle cell energetics. *Atherosclerosis* 2018;**268**:1–11.
180. Nahapetyan H, Moulis M, Grousset E, Faccini J, Grazide MH, Mucher E, Elbaz M, Martinet W, Vindis C. Altered mitochondrial quality control in Atg7-deficient VSMCs promotes enhanced apoptosis and is linked to unstable atherosclerotic plaque phenotype. *Cell Death Dis* 2019;**10**:119.
181. Clement M, Chappell J, Raffort J, Lareyre F, Vandestienne M, Taylor AL, Finigan A, Harrison J, Bennett MR, Bruneval P, Taleb S, Jorgensen HF, Mallat Z. Vascular smooth muscle cell plasticity and autophagy in dissecting aortic aneurysms. *Arterioscler Thromb Vasc Biol* 2019;**39**:1149–1159.
182. De Munck DG, Leloup AJA, De Meyer GRY, Martinet W, Fransen P. Defective autophagy in vascular smooth muscle cells increases passive stiffness of the mouse aortic vessel wall. *Pflugers Arch* 2020;**472**:1031–1040.
183. Zhang H, Ge S, He K, Zhao X, Wu Y, Shao Y, Wu X. FoxO1 inhibits autophagosome-lysosome fusion leading to endothelial autophagic-apoptosis in diabetes. *Cardiovasc Res* 2019;**115**:2008–2020.
184. Sprott D, Poitz DM, Korovina I, Ziogas A, Phieler J, Chatzigeorgiou A, Mitroulis I, Deussen A, Chavakis T, Klotzsche-von Ameln A. Endothelial-specific deficiency of ATG5 (autophagy protein 5) attenuates ischemia-related angiogenesis. *Arterioscler Thromb Vasc Biol* 2019;**39**:1137–1148.
185. Locatelli L, Cazzaniga A, De Palma C, Castiglioni S, Maier JAM. Mitophagy contributes to endothelial adaptation to simulated microgravity. *FASEB J* 2020;**34**:1833–1845.
186. De Munck DG, De Moudt S, Roth L, De Meyer GRY, Martinet W, Fransen P. Defective autophagy in vascular smooth muscle cells alters vascular reactivity of the mouse femoral artery. *Front Physiol* 2020;**11**:548943.
187. Takagaki Y, Lee SM, Dongqing Z, Kitada M, Kanasaki K, Koya D. Endothelial autophagy deficiency induces IL6-dependent endothelial mesenchymal transition and organ fibrosis. *Autophagy* 2020;**16**:1905–1914.
188. Yuan P, Hu Q, He X, Long Y, Song X, Wu F, He Y, Zhou X. Laminar flow inhibits the Hippo/YAP pathway via autophagy and SIRT1-mediated deacetylation against atherosclerosis. *Cell Death Dis* 2020;**11**:141.
189. Jeong IH, Bae WY, Choi JS, Jeong JW. Ischemia induces autophagy of endothelial cells and stimulates angiogenic effects in a hindlimb ischemia mouse model. *Cell Death Dis* 2020;**11**:624.
190. Taegtmeyer H. Energy metabolism of the heart: from basic concepts to clinical applications. *Curr Probl Cardiol* 1994;**19**:59–113.
191. Lopez-Crisosto C, Pennanen C, Vasquez-Trincado C, Morales PE, Bravo-Sagua R, Quest AFG, Chiong M, Lavandero S. Sarcoplasmic reticulum-mitochondria communication in cardiovascular pathophysiology. *Nat Rev Cardiol* 2017;**14**:342–360.
192. Saito T, Nah J, Oka SI, Mukai R, Monden Y, Maejima Y, Ikeda Y, Sciarretta S, Liu T, Li H, Baljinnyam E, Fraidenraich D, Fritzky L, Zhai P, Ichinose S, Isobe M, Hsu CP, Kundu M, Sadoshima J. An alternative mitophagy pathway mediated by Rab9 protects the heart against ischemia. *J Clin Invest* 2019;**129**:802–819.
193. Wang B, Nie J, Wu L, Hu Y, Wen Z, Dong L, Zou MH, Chen C, Wang DW. AMPKalpha2 protects against the development of heart failure by enhancing mitophagy via PINK1 phosphorylation. *Circ Res* 2018;**122**:712–729.
194. Tahrir FG, Knezevic T, Gupta MK, Gordon J, Cheung JY, Feldman AM, Khalili K. Evidence for the role of BAG3 in mitochondrial quality control in cardiomyocytes. *J Cell Physiol* 2017;**232**:797–805.
195. Rodriguez AE, Lopez-Crisosto C, Pena-Oyarzun D, Salas D, Parra V, Quiroga C, Morawe T, Chiong M, Behl C, Lavandero S. BAG3 regulates total MAP1LC3B protein levels through a translational but not transcriptional mechanism. *Autophagy* 2016;**12**:287–296.
196. Knezevic T, Myers VD, Su F, Wang J, Song J, Zhang XQ, Gao E, Gao G, Muniswamy M, Gupta MK, Gordon J, Weiner KN, Rabinowitz J, Ramsey FV, Tilley DG, Khalili K, Cheung JY, Feldman AM. Adeno-associated virus serotype 9-driven expression of BAG3 improves left ventricular function in murine hearts with left ventricular dysfunction secondary to a myocardial infarction. *JACC Basic Transl Sci* 2016;**1**:647–656.
197. Jin HS, Hong KW, Kim BY, Kim J, Yoo YH, Oh B, Jeong SY. Replicated association between genetic variation in the PARK2 gene and blood pressure. *Clin Chim Acta* 2011;**412**:1673–1677.
198. Eisenberg T, Abdellatif M, Zimmermann A, Schroeder S, Pendl T, Harger A, Stekovic S, Schipke J, Magnes C, Schmidt A, Ruckenstuhl C, Dammbrueck C, Gross AS, Herbst V, Carmona-Gutierrez D, Pietrocola F, Pieber TR, Sigrist SJ, Linke WA, Muhlfeld C, Sadoshima J, Dengjel J, Kiechl S, Kroemer G, Sedej S, Madeo F. Dietary spermidine for lowering high blood pressure. *Autophagy* 2017;**13**:767–769.
199. Valentim L, Laurence KM, Townsend PA, Carroll CJ, Soond S, Scarabelli TM, Knight RA, Latchman DS, Stephanou A. Urocortin inhibits Beclin1-mediated autophagic cell death in cardiac myocytes exposed to ischaemia/reperfusion injury. *J Mol Cell Cardiol* 2006;**40**:846–852.
200. Ma X, Liu H, Foyil SR, Godar RJ, Weinheimer CJ, Diwan A. Autophagy is impaired in cardiac ischemia-reperfusion injury. *Autophagy* 2012;**8**:1394–1396.
201. Huang L, Dai K, Chen M, Zhou W, Wang X, Chen J, Zhou W. The AMPK agonist PT1 and mTOR inhibitor 3HOI-BA-01 protect cardiomyocytes after ischemia through induction of autophagy. *J Cardiovasc Pharmacol Ther* 2016;**21**:70–81.
202. Li S, Liu C, Gu L, Wang L, Shang Y, Liu Q, Wan J, Shi J, Wang F, Xu Z, Ji G, Li W. Autophagy protects cardiomyocytes from the myocardial ischaemia-reperfusion injury through the clearance of CLP36. *Open Biol* 2016;**6**:160177.
203. Nah J, Zhai P, Huang CY, Fernandez AF, Mareedu S, Levine B, Sadoshima J. Upregulation of Rubicon promotes autosis during myocardial ischemia/reperfusion injury. *J Clin Invest* 2020;**130**:2978–2991.
204. Wu S, Chang G, Gao L, Jiang D, Wang L, Li G, Luo X, Qin S, Guo X, Zhang D. Trimetazidine protects against myocardial ischemia/reperfusion injury by inhibiting excessive autophagy. *J Mol Med (Berl)* 2018;**96**:791–806.
205. Norambuena-Soto I, Núñez-Soto C, Sanhueza-Olivares F, Cancino-Arenas N, Mondaca-Ruff D, Vivar R, Díaz-Araya G, Mellado R, Chiong M. Transforming growth factor-beta and Forkhead box O transcription factors as cardiac fibroblast regulators. *Biosci Trends* 2017;**11**:154–162.
206. Gupta SS, Zeglinski MR, Rattan SG, Landry NM, Ghavami S, Wigle JT, Klonisch T, Halayko AJ, Dixon IM. Inhibition of autophagy inhibits the conversion of cardiac fibroblasts to cardiac myofibroblasts. *Oncotarget* 2016;**7**:78516–78531.

207. Ghavami S, Cunnington RH, Gupta S, Yeganeh B, Filomeno KL, Freed DH, Chen S, Klonisch T, Halayko AJ, Ambrose E, Singal R, Dixon IM. Autophagy is a regulator of TGF-beta1-induced fibrogenesis in primary human atrial myofibroblasts. *Cell Death Dis* 2015;**6**:e1696.
208. Gao H, Yang Q, Dong R, Hou F, Wu Y. Sequential changes in autophagy in diabetic cardiac fibrosis. *Mol Med Rep* 2016;**13**:327–332.
209. Wang H, Yang X, Yang Q, Gong L, Xu H, Wu Z. PARP-1 inhibition attenuates cardiac fibrosis induced by myocardial infarction through regulating autophagy. *Biochem Biophys Res Commun* 2018;**503**:1625–1632.
210. Westermeier F, Riquelme JA, Pavez M, Garrido V, Diaz A, Verdejo HE, Castro PF, Garcia L, Lavandero S. New molecular insights of insulin in diabetic cardiomyopathy. *Front Physiol* 2016;**7**:125.
211. Munasinghe PE, Riu F, Dixit P, Edamatsu M, Saxena P, Hamer NS, Galvin IF, Bunton RW, Lequeux S, Jones G, Lamberts RR, Emanueli C, Madeddu P, Katare R. Type-2 diabetes increases autophagy in the human heart through promotion of Beclin-1 mediated pathway. *Int J Cardiol* 2016;**202**:13–20.
212. Durga Devi T, Babu M, Mäkinen P, Kaikkonen MU, Heinaniemi M, Laakso H, Ylä-Herttuala E, Rieppo L, Liimatainen T, Naumenko N, Tavi P, Ylä-Herttuala S. Aggravated postinfarct heart failure in type 2 diabetes is associated with impaired mitophagy and exaggerated inflammasome activation. *Am J Pathol* 2017;**187**: 2659–2673.
213. Mariappan N, Elks CM, Sriramula S, Guggilam A, Liu Z, Borkhsenious O, Francis J. NF-kappaB-induced oxidative stress contributes to mitochondrial and cardiac dysfunction in type II diabetes. *Cardiovasc Res* 2010;**85**:473–483.
214. Kanamori H, Takemura G, Goto K, Tsujimoto A, Mikami A, Ogino A, Watanabe T, Morishita K, Okada H, Kawasaki M, Seishima M, Minatoguchi S. Autophagic adaptations in diabetic cardiomyopathy differ between type 1 and type 2 diabetes. *Autophagy* 2015;**11**:1146–1160.
215. Andres AM, Kooren JA, Parker SJ, Tucker KC, Ravindran N, Ito BR, Huang C, Venkatraman V, Van Eyk JE, Gottlieb RA, Mentzer RM Jr. Discordant signaling and autophagy response to fasting in hearts of obese mice: implications for ischemia tolerance. *Am J Physiol Heart Circ Physiol* 2016;**311**:H219–H228.
216. Chandramouli C, Reichelt ME, Curl CL, Varma U, Bienvenu LA, Koutsifeli P, Raaijmakers AJA, De Blasio MJ, Qin CX, Jenkins AJ, Ritchie RH, Mellor KM, Delbridge LMD. Diastolic dysfunction is more apparent in STZ-induced diabetic female mice, despite less pronounced hyperglycemia. *Sci Rep* 2018;**8**:2346.
217. Wende AR, Schell JC, Ha CM, Pepin ME, Khalimonchuk O, Schwertz H, Pereira RO, Brahma MK, Tuinei J, Contreras-Ferrat A, Wang L, Andrizzi CA, Olsen CD, Bradley WE, Dell'Italia LJ, Dillmann WH, Litwin SE, Abel ED. Maintaining myocardial glucose utilization in diabetic cardiomyopathy accelerates mitochondrial dysfunction. *Diabetes* 2020;**69**:2094–2111.
218. Yuan X, Xiao YC, Zhang GP, Hou N, Wu XQ, Chen WL, Luo JD, Zhang GS. Chloroquine improves left ventricle diastolic function in streptozotocin-induced diabetic mice. *Drug Des Devel Ther* 2016;**10**:2729–2737.
219. Kobayashi S, Patel J, Zhao F, Huang Y, Kobayashi T, Liang Q. Novel dual-fluorescent mitophagy reporter reveals a reduced mitophagy flux in type 1 diabetic mouse heart. *J Am Osteopath Assoc* 2020;**120**:446–455.
220. Xu X, Kobayashi S, Chen K, Timm D, Volden P, Huang Y, Gulick J, Yue Z, Robbins J, Epstein PN, Liang Q. Diminished autophagy limits cardiac injury in mouse models of type 1 diabetes. *J Biol Chem* 2013;**288**:18077–18092.
221. Wei H, Qu H, Wang H, Ji B, Ding Y, Liu D, Duan Y, Liang H, Peng C, Xiao X, Deng H. 1,25-Dihydroxyvitamin-D3 prevents the development of diabetic cardiomyopathy in type 1 diabetic rats by enhancing autophagy via inhibiting the beta-catenin/TCF4/GSK-3beta/mTOR pathway. *J Steroid Biochem Mol Biol* 2017;**168**:71–90.
222. Wang B, Yang Q, Sun YY, Xing YF, Wang YB, Lu XT, Bai WW, Liu XQ, Zhao YX. Resveratrol-enhanced autophagic flux ameliorates myocardial oxidative stress injury in diabetic mice. *J Cell Mol Med* 2014;**18**:1599–1611.
223. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; ESC Scientific Document Group. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016;**37**:2129–2200.
224. Redfield MM. Heart failure with preserved ejection fraction. *N Engl J Med* 2016;**375**: 1868–1877.
225. Gladden JD, Linke WA, Redfield MM. Heart failure with preserved ejection fraction. *Pflugers Arch* 2014;**466**:1037–1053.
226. Hahn VS, Knutsdottir H, Luo X, Bedi K, Margulies KB, Haldar SM, Stolina M, Yin J, Khakoo AY, Vaishnav J, Bader JS, Kass DA, Sharma K. Myocardial gene expression signatures in human heart failure with preserved ejection fraction. *Circulation* 2021; **143**:120–134.
227. Zhang C, Liu A, Su G, Chen Y. Effect of rapamycin on the level of autophagy in rats with early heart failure. *J Cell Biochem* 2019;**120**:4065–4070.
228. Chiao YA, Kolwicz SC, Basisty N, Gagnidze A, Zhang J, Gu H, Djukovic D, Beyer RP, Raftery D, MacCoss M, Tian R, Rabinovitch PS. Rapamycin transiently induces mitochondrial remodeling to reprogram energy metabolism in old hearts. *Aging (Albany NY)* 2016;**8**:314–327.
229. Gao G, Chen W, Yan M, Liu J, Luo H, Wang C, Yang P. Rapamycin regulates the balance between cardiomyocyte apoptosis and autophagy in chronic heart failure by inhibiting mTOR signaling. *Int J Mol Med* 2020;**45**:195–209.
230. Nandi SS, Katsurada K, Sharma NM, Anderson DR, Mahata SK, Patel KP. MMP9 inhibition increases autophagic flux in chronic heart failure. *Am J Physiol Heart Circ Physiol* 2020;**319**:H1414–H1437.
231. Ranek MJ, Kokkonen-Simon KM, Chen A, Dunkerly-Eyring BL, Vera MP, Oeing CU, Patel CH, Nakamura T, Zhu G, Bedja D, Sasaki M, Holewinski RJ, Van Eyk JE, Powell JD, Lee DI, Kass DA. PKG1-modified TSC2 regulates mTORC1 activity to counter adverse cardiac stress. *Nature* 2019;**566**:264–269.
232. Saito T, Asai K, Sato S, Hayashi M, Adachi A, Sasaki Y, Takano H, Mizuno K, Shimizu W. Autophagic vacuoles in cardiomyocytes of dilated cardiomyopathy with initially decompensated heart failure predict improved prognosis. *Autophagy* 2016;**12**: 579–587.
233. Wu P, Yuan X, Li F, Zhang J, Zhu W, Wei M, Li J, Wang X. Myocardial upregulation of cathepsin D by ischemic heart disease promotes autophagic flux and protects against cardiac remodeling and heart failure. *Circ Heart Fail* 2017;**10**:e004044.
234. Corsetti G, Chen-Scarabelli C, Romano C, Pasini E, Dioguardi FS, Onorati F, Knight R, Patel H, Saravolatz L, Faggian G, Scarabelli TM. Autophagy and oncosis/necroptosis are enhanced in cardiomyocytes from heart failure patients. *Med Sci Monit Basic Res* 2019;**25**:33–44.
235. Zhang H, Yin Y, Liu Y, Zou G, Huang H, Qian P, Zhang G, Zhang J. Necroptosis mediated by impaired autophagy flux contributes to adverse ventricular remodeling after myocardial infarction. *Biochem Pharmacol* 2020;**175**:113915.
236. Gao T, Zhang SP, Wang JF, Liu L, Wang Y, Cao ZY, Hu QK, Yuan WJ, Lin L. TLR3 contributes to persistent autophagy and heart failure in mice after myocardial infarction. *J Cell Mol Med* 2018;**22**:395–408.
237. Li B, Chi RF, Qin FZ, Guo XF. Distinct changes of myocyte autophagy during myocardial hypertrophy and heart failure: association with oxidative stress. *Exp Physiol* 2016;**101**:1050–1063.
238. Li Y, Wang Y, Zou M, Chen C, Chen Y, Xue R, Dong Y, Liu C. AMPK blunts chronic heart failure by inhibiting autophagy. *Biosci Rep* 2018;**38**:BSR20170982.
239. Shirakabe A, Ikeda Y, Sciarretta S, Zablocki DK, Sadoshima J. Aging and autophagy in the heart. *Circ Res* 2016;**118**:1563–1576.
240. Taneike M, Yamaguchi O, Nakai A, Hikoso S, Takeda T, Mizote I, Oka T, Tamai T, Oyabu J, Murakawa T, Nishida K, Shimizu T, Hori M, Komuro I, Takuji Shirasawa TS, Mizushima N, Otsu K. Inhibition of autophagy in the heart induces age-related cardiomyopathy. *Autophagy* 2010;**6**:600–606.
241. Khalil H, Tazi M, Caution K, Ahmed A, Kanneganti A, Assani K, Kopp B, Marsh C, Dakhlallah D, Amer AO. Aging is associated with hypermethylation of autophagy genes in macrophages. *Epigenetics* 2016;**11**:381–388.
242. Fernandez AF, Sebti S, Wei Y, Zou Z, Shi M, McMillan KL, He C, Ting T, Liu Y, Chiang WC, Marciano DK, Schiattarella GG, Bhagat G, Moe OW, Hu MC, Levine B. Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. *Nature* 2018;**558**:136–140.
243. Chavez MN, Morales RA, Lopez-Crisosto C, Roa JC, Allende ML, Lavandero S. Autophagy activation in zebrafish heart regeneration. *Sci Rep* 2020;**10**:2191.
244. Chiong M, Cartes-Saavedra B, Norambuena-Soto I, Mondaca-Ruff D, Morales PE, García-Miguel M, Mellado R. Mitochondrial metabolism and the control of vascular smooth muscle cell proliferation. *Front Cell Dev Biol* 2014;**2**:72.
245. Liu Y, Liu B, Li Y, Li Y, Du J, Deng S, Jing X, She Q. Autophagy is involved in the differentiation of epicardial progenitor cells into vascular smooth muscle cells in mice. *Exp Cell Res* 2019;**375**:60–71.
246. Garrido-Moreno V, Díaz-Vegas A, López-Crisosto C, Troncoso MF, Navarro-Marquez M, García L, Estrada M, Cifuentes M, Lavandero S. GDF-11 prevents cardiomyocyte hypertrophy by maintaining the sarcoplasmic reticulum-mitochondria communication. *Pharmacol Res* 2019;**146**:104273.
247. Yuan X, Bhat OM, Lohner H, Li N, Zhang Y, Li PL. Inhibitory effects of growth differentiation factor 11 on autophagy deficiency-induced dedifferentiation of arterial smooth muscle cells. *Am J Physiol Heart Circ Physiol* 2019;**316**:H345–H356.
248. Mondaca-Ruff D, Riquelme JA, Quiroga C, Norambuena-Soto I, Sanhueza-Olivares F, Villar-Fincheira P, Hernandez-Diaz T, Cancino-Arenas N, San Martin A, Garcia L, Lavandero S, Chiong M. Angiotensin II-regulated autophagy is required for vascular smooth muscle cell hypertrophy. *Front Pharmacol* 2018;**9**:1553.
249. Wang N, Xu F, Lu S, Zhang N, Sun Y. Septin4 as an autophagy modulator regulates Angiotensin-II mediated VSMCs proliferation and migration. *Biochem Biophys Res Commun* 2020;**525**:272–279.
250. Wang Y, Zhang X, Chen W, Gao L, Li J, Song T, Chi J, Zhang X, Shi Z, Dong Y, Yin X, Liu Y. Cortistatin ameliorates Ang II-induced proliferation of vascular smooth muscle cells by inhibiting autophagy through SSTR3 and SSTR5. *Life Sci* 2020;**253**: 117726.
251. Li T, Tan X, Zhu S, Zhong W, Huang B, Sun J, Li F, Wang Y. SPARC induces phenotypic modulation of human brain vascular smooth muscle cells via AMPK/mTOR-mediated autophagy. *Neurosci Lett* 2019;**712**:134485.
252. Ouyang C, Mu J, Lu Q, Li J, Zhu H, Wang Q, Zou MH, Xie Z. Autophagic degradation of KAT2A/GCN5 promotes directional migration of vascular smooth muscle cells by reducing TUBA/alpha-tubulin acetylation. *Autophagy* 2019;**16**:1753–1770.
253. Chatterjee S. Endothelial mechanotransduction, redox signaling and the regulation of vascular inflammatory pathways. *Front Physiol* 2018;**9**:524.

254. Liu J, Bi X, Chen T, Zhang Q, Wang SX, Chiu JJ, Liu GS, Zhang Y, Bu P, Jiang F. Shear stress regulates endothelial cell autophagy via redox regulation and Sirt1 expression. *Cell Death Dis* 2015;**6**:e1827.
255. Zhang JX, Qu XL, Chu P, Xie DJ, Zhu LL, Chao YL, Li L, Zhang JJ, Chen SL. Low shear stress induces vascular eNOS uncoupling via autophagy-mediated eNOS phosphorylation. *Biochim Biophys Acta Mol Cell Res* 2018;**1865**:709–720.
256. Park SK, La Salle DT, Cerbie J, Cho JM, Bledsoe A, Nelson A, Morgan DE, Richardson RS, Shiu YT, Boudina S, Trinity JD, Symons JD. Elevated arterial shear rate increases indexes of endothelial cell autophagy and nitric oxide synthase activation in humans. *Am J Physiol Heart Circ Physiol* 2019;**316**:H106–H112.
257. Renna NF, de Las Heras N, Miatello RM. Pathophysiology of vascular remodeling in hypertension. *Int J Hypertens* 2013;**2013**:808353.
258. Campbell GR, Campbell JH. Smooth muscle phenotypic changes in arterial wall homeostasis: implications for the pathogenesis of atherosclerosis. *Exp Mol Pathol* 1985; **42**:139–162.
259. Cecchettini A, Rocchiccioli S, Boccardi C, Citti L. Vascular smooth-muscle-cell activation: proteomics point of view. *Int Rev Cell Mol Biol* 2011;**288**:43–99.
260. Kim Y, Park JK, Seo JH, Ryu HS, Lim KS, Jeong MH, Kang DH, Kang SW. A rapamycin derivative, biolimus, preferentially activates autophagy in vascular smooth muscle cells. *Sci Rep* 2018;**8**:16551.
261. Osonoi Y, Mita T, Azuma K, Nakajima K, Masuyama A, Goto H, Nishida Y, Miyatsuka T, Fujitani Y, Koike M, Mitsumata M, Watada H. Defective autophagy in vascular smooth muscle cells enhances cell death and atherosclerosis. *Autophagy* 2018;**14**:1991–2006.
262. Ramadan A, Singh KK, Quan A, Plant PJ, Al-Omran M, Teoh H, Verma S. Loss of vascular smooth muscle cell autophagy exacerbates angiotensin II-associated aortic remodeling. *J Vasc Surg* 2018;**68**:859–871.
263. Lu H, Sun J, Liang W, Chang Z, Rom O, Zhao Y, Zhao G, Xiong W, Wang H, Zhu T, Guo Y, Chang L, Garcia-Barrio MT, Zhang J, Chen YE, Fan Y. Cyclodextrin prevents abdominal aortic aneurysm via activation of vascular smooth muscle cell transcription factor EB. *Circulation* 2020;**142**:483–498.
264. Wang Z, Guo J, Han X, Xue M, Wang W, Mi L, Sheng Y, Ma C, Wu J, Wu X. Metformin represses the pathophysiology of AAA by suppressing the activation of PI3K/AKT/mTOR/autophagy pathway in ApoE(-/-) mice. *Cell Biosci* 2019;**9**:68.
265. Wu QY, Cheng Z, Zhou YZ, Zhao Y, Li JM, Zhou XM, Peng HL, Zhang GS, Liao XB, Fu XM. A novel STAT3 inhibitor attenuates angiotensin II-induced abdominal aortic aneurysm progression in mice through modulating vascular inflammation and autophagy. *Cell Death Dis* 2020;**11**:131.
266. Pi S, Mao L, Chen J, Shi H, Liu Y, Guo X, Li Y, Zhou L, He H, Yu C, Liu J, Dang Y, Xia Y, He Q, Li JH, Hu Y, Miao Y, Yue Y, Hu B. The P2RY12 receptor promotes VSMC-derived foam cell formation by inhibiting autophagy in advanced atherosclerosis. *Autophagy* 2020;**17**:980–1000.
267. Masuyama A, Mita T, Azuma K, Osonoi Y, Nakajima K, Goto H, Nishida Y, Miyatsuka T, Mitsumata M, Watada H. Defective autophagy in vascular smooth muscle cells enhances atherosclerotic plaque instability. *Biochem Biophys Res Commun* 2018;**505**:1141–1147.
268. Evans TD, Jeong SJ, Zhang X, Sergin I, Razani B. TFEB and trehalose drive the macrophage autophagy-lysosome system to protect against atherosclerosis. *Autophagy* 2018;**14**:724–726.
269. Yuan X, Bhat OM, Meng N, Lohner H, Li PL. Protective role of autophagy in Nlrp3 inflammasome activation and medial thickening of mouse coronary arteries. *Am J Pathol* 2018;**188**:2948–2959.
270. Chen R, Jiang M, Li B, Zhong W, Wang Z, Yuan W, Yan J. The role of autophagy in pulmonary hypertension: a double-edge sword. *Apoptosis* 2018;**23**:459–469.
271. Gomez-Puerto MC, van Zuijen I, Huang CJ, Szulcek R, Pan X, van Dinther MA, Kurakula K, Wiesmeijer CC, Goumans M-J, Bogaard H-J, Morrell NW, Rana AA, Ten Dijke P. Autophagy contributes to BMP type 2 receptor degradation and development of pulmonary arterial hypertension. *J Pathol* 2019;**249**:356–367.
272. Guo L, Li Y, Tian Y, Gong S, Chen X, Peng T, Wang A, Jiang Z. eIF2alpha promotes vascular remodeling via autophagy in monocrotaline-induced pulmonary arterial hypertension rats. *Drug Des Devel Ther* 2019;**13**:2799–2809.
273. Feng W, Wang J, Yan X, Zhai C, Shi W, Wang Q, Zhang Q, Li M. Paclitaxel alleviates monocrotaline-induced pulmonary arterial hypertension via inhibition of FoxO1-mediated autophagy. *Naunyn Schmiedebergs Arch Pharmacol* 2019;**392**: 605–613.