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NARRATIVE REVIEW

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Mitophagy modulation for the treatment of cardiovascular diseases

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Abstract

Background: Defects of mitophagy, the selective form of autophagy for mitochondria, are commonly observed in several cardiovascular diseases and represent the main cause of mitochondrial dysfunction. For this reason, mitophagy has emerged as a novel and potential therapeutic target.

Methods: In this review, we discuss current evidence about the biological significance of mitophagy in relevant preclinical models of cardiac and vascular

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diseases, such as heart failure, ischemia/reperfusion injury, metabolic cardiomyopathy and atherosclerosis.

Results: Multiple studies have shown that cardiac and vascular mitophagy is an adaptive mechanism in response to stress, contributing to cardiovascular homeostasis. Mitophagy defects lead to cell death, ultimately impairing cardiac and vascular function, whereas restoration of mitophagy by specific compounds delays disease progression.

Conclusions: Despite previous efforts, the molecular mechanisms underlying mitophagy activation in response to stress are not fully characterized. A comprehensive understanding of different forms of mitophagy active in the cardiovascular system is extremely important for the development of new drugs targeting this process. Human studies evaluating mitophagy abnormalities in patients at high cardiovascular risk also represent a future challenge.

KEYWORDS

autophagy, heart failure, metabolic cardiomyopathy, mitochondrial dysfunction, mitophagy, myocardial ischemia

1 | INTRODUCTION

Mitochondria are fundamental organelles that convert chemical energy derived from oxidation of nutrients to catalyse the phosphorylation of adenosine diphosphate (ADP) to form adenosine triphosphate (ATP), which supports cardiac contraction and relaxation.[1,2] For this reason, mitochondrial health is continuously monitored by various quality-control mechanisms. Dysfunctional mitochondria represent the major cellular source of reactive oxygen species (ROS) and the main trigger of cell death mechanisms.[2] Several lines of evidence suggest that mitochondrial dysfunction is a determinant of multiple cardiovascular diseases (CVDs), due to the depletion of ATP and the increase in oxidative stress, which are important contributors of cardiac and vascular dysfunction.[1]

Therapies targeting mitochondria were shown to exert beneficial effects in preclinical models of CVDs and several compounds, such as mitochondria-targeted antioxidant elamipretide or the cell permeable peptide SS-31 are already being tested in clinical trials.[3]

Mitochondrial homeostasis is ensured by different mechanisms including biogenesis, mitochondrial dynamics (fusion and fission) and mitophagy (Figure 1).[4–6] Mitochondria undergo coordinated cycles of fusion and fission under basal conditions or in response to mitochondrial stress, such as changes of mitochondrial membrane potential ($\Delta\Psi$m) or nutrient and oxygen depletion.[5] Generally, fusion is activated in the presence of reversible mitochondrial damage, while mitochondrial fission occurs when irreversibly damaged mitochondria accumulate.[5] Elongated mitochondria are the result of fusion activity, whereas fragmented and small-spheroid mitochondria are produced by fission. Mitofusin 1 and 2 (Mfn1-2) and optic atrophy 1 protein (Opa1) represent the main orchestrators of mitochondrial fusion, allowing the fusion between the outer (OMM) and inner mitochondrial membranes (IMM).[5,7] Dynamin-related protein 1 (Drp1), mitochondrial fission 1 protein (Fis1), mitochondrial division protein 1 (Mdv1) and mitochondrial fission factor (Mff) are instead involved in mitochondrial fission. Damaged and aged mitochondria, including those derived from mitochondrial fission, can be removed by mitophagy and replaced by new freshly formed mitochondria by biogenesis.[7]

Mitophagy is a cargo-specific form of autophagy devoted to the degradation of dysfunctional, damaged or aged mitochondria.[8,9] Autophagy is an evolutionarily conserved intracellular catabolic process, which removes dysfunctional cellular elements.[10] Apart from its relevance in basal conditions, autophagy represents a crucial adaptive mechanism for the cell in response to stress conditions, limiting damage and cell death.[10] The main form of autophagy, called macroautophagy, consists of a regulated series of events: the cytoplasmic cargo is initially engulfed by a double-membrane vesicle called autophagosome and then delivered to the lysosome for degradation, resulting in the formation of the autolysosome.[10] Autophagy acts as the main mechanism that

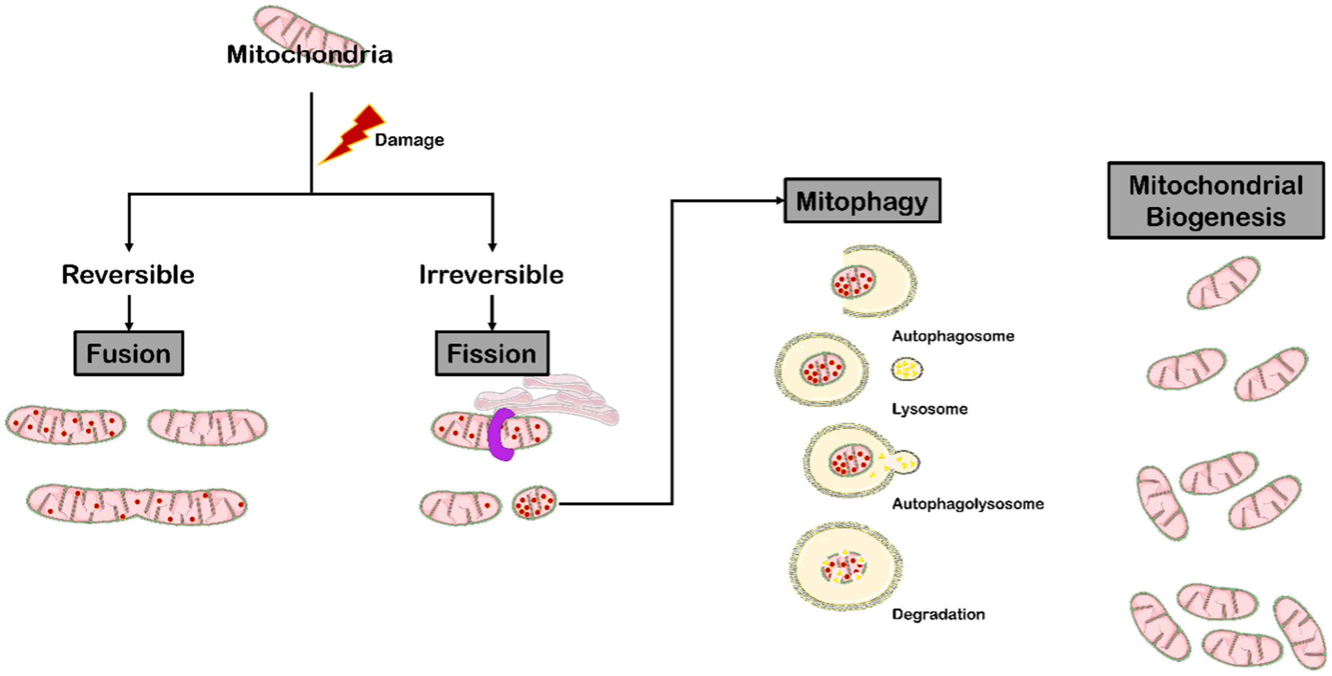


FIGURE 1 Mechanisms regulating mitochondrial homeostasis. Mitochondria undergo coordinated cycles of fusion and fission in response to damage (left). Aged or dysfunctional mitochondria are removed by mitophagy (central panel) and replaced by new mitochondria produced by biogenesis (right). Elements were modified from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic Licence. https://smart.servier.com/; https://creativecommons.org/licenses/by/3.0/

regulates cardiac and vascular homeostasis.[10,11] It is generally activated during pressure overload, exerting adaptive mechanisms that improve cardiac remodelling and limit cardiac damage. However, if excessively activated, such as during the reperfusion phase after an ischemic episode, autophagy can trigger maladaptive mechanisms, exacerbating cardiac injury.[11] Autophagy also decreases during ageing and in response to metabolic alterations, leading to an increase in cardiac complications.[12,13] Mitochondrial-specific autophagy, namely mitophagy, also plays a pivotal role in the regulation of cardiovascular homeostasis, preserving cardiac function at baseline and conferring myocardial protection in response to stress.[14] Adaptive effects induced by mitophagy in response to cardiac stress include the removal of injured mitochondria. The latter preserves mitochondrial function, by maintaining both energy production and redox status and inhibiting cell death mechanisms. Interestingly, previous studies demonstrated that timing and duration of mitophagy activation in response to cardiac stress are different and often not correlated with respect to stress-induced general autophagy, suggesting that these processes are regulated by different mechanisms and involve a different machinery.[15]

In this review, we discuss preclinical studies analysing the role of mitophagy in models of CVDs, both in the heart and in the vascular system (Table 1). We also highlight fundamental evidence suggesting the translational relevance of mitophagy as potential therapeutic target for CVDs.

2 | BIOLOGY OF MITOPHAGY

2.1 | Parkin-dependent mitophagy

Mitophagy can be sub-classified into two main forms, named Parkin-dependent or Parkin-independent mitophagy (Figure 2).

Parkin-dependent mitophagy involves the accumulation of the serine/threonine kinase phosphatase and tensin homologue (Pten)-induced kinase 1 (Pink1) on the OMM in response to $\Delta\Psi m$ depolarization or mitochondrial unfolded protein response (UPRmt) activation.[28,29] Conversely, Pink1 localizes at the IMM in unstressed conditions and is degraded by the mitochondrial processing peptidase (Mpp) and the Presenilin-associated rhomboid-like (Parl).[30,31] After stress-induced accumulation, Pink1 recruits Parkin on the cytosolic face of the OMM.[31] Parkin is an E3-ubiquitin ligase which ubiquitinates several components of the OMM, allowing their interaction with mitophagy receptors/adaptors, such as p62/sequestosome or neighbour of Brca1 (NBR1), which in turn interact with microtubule-associated proteins 1A/1B light chain 3B (LC3) and then promote autophagosome formation and mitochondria engulfment.[32]

TABLE 1 Cardiac effects of genetic inhibition of mitophagy during stress conditions.

Mouse Model	Condition	Age and gender	Cardiac phenotype	Endpoint parameter	Reference
Transgenic mice overexpressing Parkin	Ageing	Age: 20-weeks Gender: male	↑ cardiac function ↓ cardiac ageing	Increase in Maximum dP/dt $>2\times10^{-3}$ mmHg ms^{-1} Reduction of senescence-associated β-galactosidase >50%	[16]
Cardiac-specific Drp1 heterozygous knockout mice	Pressure overload (PO) induced by Transverse aortic constriction (TAC)	Age: 16-weeks Gender: not specified	↓ cardiac function ↑ Heart failure	Reduction in left ventricular ejection fraction (LVEF) >20% at 3 and 7 days after TAC	[17]
Cardiac-specific Ulk1 knockout mice	PO induced by TAC	Age: 10–16 weeks Gender: male and female	↓ cardiac function ↑ Heart failure	Reduction in LVEF >20% from 3 to 30 days after TAC	[18]
Systemic Parkin knockout mice	Myocardial infarction (MI) By permanent ligation of the left anterior descending coronary artery (LAD)	Age: 10–12 weeks Gender: not specified	↓ cardiac function ↑ Infarct size	Reduction in fractional shortening (FS) and ejection fraction (EF) >20%; Increase in ventricular remodelling >20% (% of total LV)	[19]
Mutant Rab mice	2 h MI by ligation of the LAD	Age: 8–12 weeks Gender: not specified	↑ Infarct size	Increase in infarct size >25%	[20]
Cardiac-specific Drp1 heterozygous knockout mice	Thirty minutes ischemia followed by 24 h reperfusion	Age: 12-weeks Gender: not specified	↑ Infarct size	Increase in infarct size >15%	[21]
Pgam5 knockout mice	Twenty five minutes ischemia followed by 90 min reperfusion in isolated hearts	Age: not specified Gender: not specified	↑ infarct size	Increase in infarct size >15%	[22]
Mutant Clock mice	Forty five minutes ischemia followed by 24 h reperfusion	Age: not specified Gender: male	↓ cardiac function	Reduction in EF >6%; Reduction in FS >4%	[23]
Systemic Parkin knockout mice	Three cycles of ischemic preconditioning followed by 20 min ischemia and 22 h of reperfusion	Age: 10–12 weeks Gender: not specified	↑ Infarct size	Increase in infarct size >20%	[24]
Systemic Parkin knockout mice	High fat diet (HFD) for 2 months to induce metabolic cardiomyopathy	Age: not specified Gender: Male and female	↑ cardiac hypertrophy ↑ diastolic dysfunction	Increase in LV weight/tibia length LW/TL >2 mg; Increase in end diastolic pressure-volume relationship (EDPVR) >0.04 mmHg/mm	[25]
Cardiac-specific Ulk1 knockout mice	HFD for 20 weeks	Age: not specified Gender: male and female	↑ diastolic and systolic dysfunction	Increase in EDPVR >0.05 mmHg/mm; reduction in FS >10%	[26]

TABLE 1 (Continued)

Mouse Model	Condition	Age and gender	Cardiac phenotype	Endpoint parameter	Reference
Mutant Rab mice	HFD for 20 weeks	Age: not specified Gender: male and female	↑ cardiac hypertrophy ↑ left ventricular dysfunction	Increase in LW/TL >2 mg; Increase in EDPVR >0.05 mmHg/mm; Reduction in LVEF >13% and in LVFS >6%	[26]
Cardiac-specific inducible Drp1 knockout mice	HFD for 3 weeks	Age: 4–12 weeks Gender: not specified	↑ diastolic and systolic dysfunction	Reduction in LVEF >13% and LEVFS >10%; Increase in LVEDD >0.30 mm	[27]

2.2 | Parkin-independent mechanisms and alternative mitophagy

Among Parkin-independent forms of mitophagy, those regulated by specific receptors play a major role. Bcl2/adenovirus E1B 19-kDa protein-interacting protein 3 (Bnip3), Nip3-like protein 13 (Nix) and FUN14 domain-containing protein 1 (Fundc1) are localized on the OMM where they can interact with LC3 (Figure 2). Bnip3 is activated during hypoxia both in cancer cells and during cardiac ischemia/reperfusion (I/R).[33–35] Fundc1 is negatively regulated by Src kinase and casein kinase 2 while it is activated by Unc-51 Like Autophagy Activating Kinase 1 (Ulk1)-mediated phosphorylation or by a dephosphorylation mediated by the diphosphatase phosphoglycerate mutase 5 (PGAM5).[36] Ulk-1 is a Ser/Thr kinase required for early autophagosome formation, both for mitophagy and for nonselective autophagy.[37]

Mitochondria removal may also occur through alternative pathways, which require neither Parkin involvement nor LC3-dependent autophagosome formation. For example, in conditions of energy stress in cardiomyocytes, mitophagy is activated by a multiprotein complex which includes Ulk1, Ras related protein Rab9, receptor-interacting serine/threonine protein kinase 1 (Rip1) and Drp1. Upon stress, activated mitogen protein kinase (AMPK) activates Ulk1 by phosphorylation at serine 555. Ulk1 in turn phosphorylates Rab9, which is localized in the membrane of autophagosomes derived from the trans-Golgi. Once phosphorylated, Rab9 interacts with Rip1, which in turn phosphorylates Drp1 at serine 616. Mitochondria harbouring the phosphorylated form of Drp1 are then sequestered by autophagosome presenting Rab9 (Figure 2). These results suggest that alternative mechanisms, which involve Ulk and Rab9-positive autophagosome, but are independent of Atg5/7 and LC3 conjugation system, play a major role in the regulation of mitophagy and adaptation to stress in cardiomyocytes.[20]

2.3 | Metabolic control of mitophagy

Mitophagy is tightly regulated by energy status and cellular metabolism.[38] The decline in ATP production and the increase of AMP/ATP ratio in response to metabolic stress represents a trigger for the activation of AMPK, which activates mitophagy as described before. In contrast, abundance of amino acids and growth factor activates the mechanistic target of rapamycin complex 1 (mTORC1), a negative regulator of autophagy.[39] It was demonstrated that in the presence of high levels of amino acids, the increase of mTOR activity in macrophages

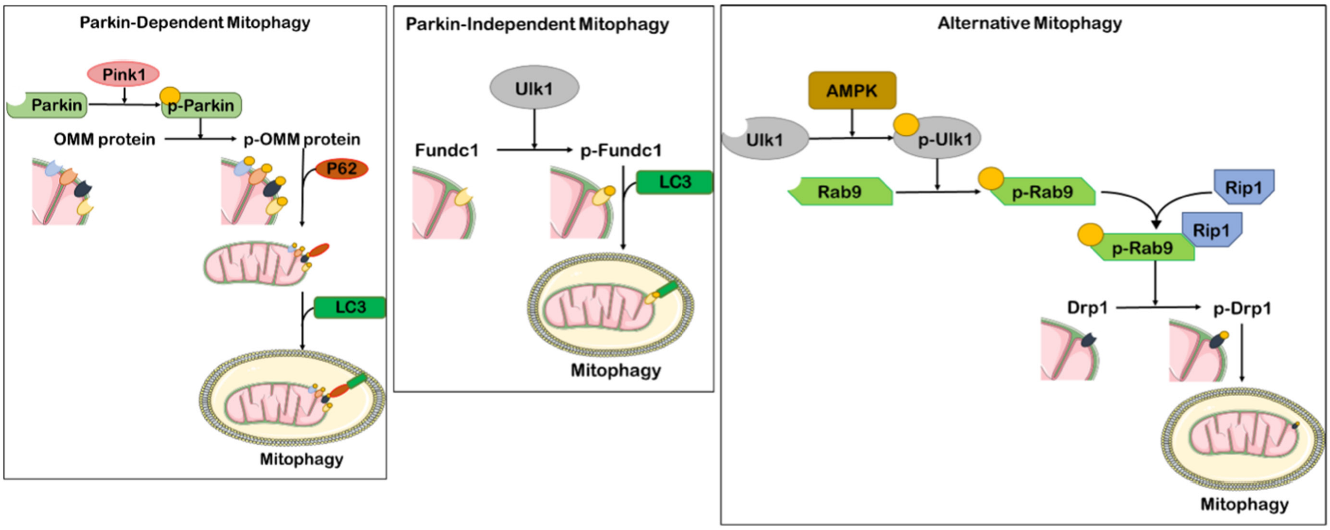


FIGURE 2 Main forms of mitophagy. Schematic representation of the molecular mechanisms involved in Parkin-dependent, -independent and alternative mitophagy. See the text for further details. Legend: AMPK, activated mitogen protein kinase; Drp1, Dynamin-related protein 1; FUNDC1, FUN14 domain-containing protein 1; LC3, microtubule-associated proteins 1A/1B light chain 3B; OMM, outer mitochondrial membrane; P62, sequestosome; Pink1, phosphatase and tensin homologue (Pten)-induced kinase 1; Rab9, Ras-related proteins; Rip1, receptor-interacting serine/threonine protein kinase 1; Ulk1, Unc-51 Like Autophagy Activating Kinase 1; p- indicates phosphorylation. Elements were modified from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic Licence. https://smart.servier.com/; https://creativecommons.org/licenses/by/3.0/

drives atherosclerosis through the inhibition of mitophagy.[40] However, the molecular mechanisms through which mTORC1 inhibits cardiac mitophagy should be clarified by further studies. Macrophages themselves also regulate mitophagy at baseline and in response to stress in the heart. Cardiomyocytes are able to deliver dysfunctional mitochondria to macrophages, through subcellular particles known as exophers, in a process mediated by the autophagic machinery. Exophers activation increases in response to cardiomyocyte stress and cardiac autophagic flux is inhibited when macrophage function is impaired. These results suggest that resident phagocytes contribute to cardiac homeostasis and autophagy.[41]

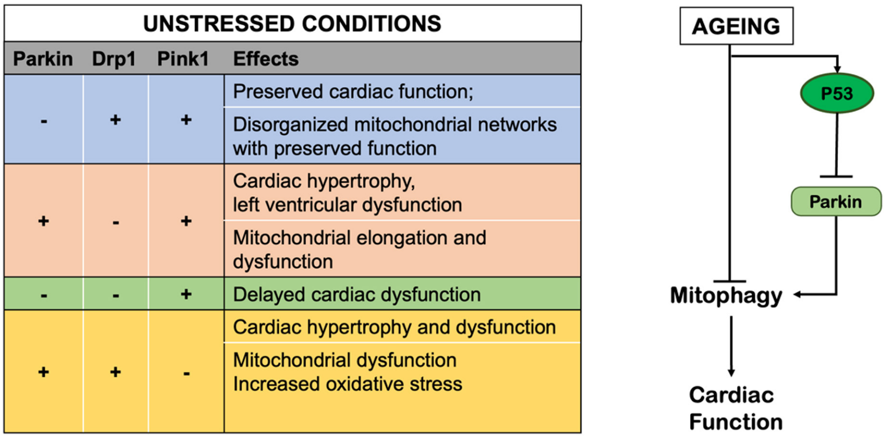
Mitophagy is also regulated by levels of acetyl-coenzyme A (CoA) and nicotinamide adenine dinucleotide (NAD$^+$). Acetil-CoA increases due to catabolic reactions of macromolecules, in the presence of nutrient abundance.[42] In this condition, it was suggested that acetyl-CoA inhibits mitophagy through the acetylation of mitochondrial proteins or through histone acetyltransferase EP300-induced acetylation of LC3.[43,44] However, the specific mechanism by which protein acetylation leads to the impairment of cardiac mitophagy remains unknown. NAD$^+$, derived from Mitochondrial Oxidative Phosphorylation System (OXPHOS) stimulates autophagy and mitophagy. NAD$^+$ supplementation with exogenous precursors exerts beneficial effects in models of ageing and CVDs.[45,46] Mechanistically, NAD$^+$ stimulates mitophagy by activating the protein deacetylase sirtuins. However, although NAD$^+$ was reported to induce nonselective autophagy through deacetylation of autophagy proteins ATG or LC3, future studies are warranted to clarify how sirtuins regulate mitophagy in response to NAD$^+$.[47]

Other evidence demonstrated that calcium influx into mitochondria, as consequence of mitochondria membrane depolarization, activates mitophagy through a Parkin-dependent mechanism and with the involvement of calcium sensors ROTH1 and ROTH2.[48] In addition, mice with cardiac specific deletion of ATG5 show mitochondria alterations along with calcium cycling abnormalities in response to beta-adrenergic stimulation with a consequent cardiac energy exhaustion, which in turn contributes to heart failure.[49] However, the specific mechanism by which autophagy/mitophagy regulates calcium cycling should be characterized. Cyclic AMP (cAMP) also regulates mitophagy by protein kinase A (PKA) activation. In this regard, cAMP-induced activation of PKA impairs mitophagy by reducing Parkin recruitment to damaged mitochondria in vitro. This evidence and its relevance in the cardiovascular system should be investigated.[50]

3 | MITOPHAGY AND HEART

3.1 | Cardiac development and ageing

Preclinical evidence obtained in animal models with cardiac-specific deletion of components of mitophagy



UNSTRESSED CONDITIONS

Parkin	Drp1	Pink1	Effects
-	+	+	Preserved cardiac function; Disorganized mitochondrial networks with preserved function
+	-	+	Cardiac hypertrophy, left ventricular dysfunction Mitochondrial elongation and dysfunction
-	-	+	Delayed cardiac dysfunction
+	+	-	Cardiac hypertrophy and dysfunction Mitochondrial dysfunction Increased oxidative stress

FIGURE 3 Role of mitophagy at baseline and during ageing. Left: Cardiac function in mice with systemic Parkin deletion (−/+/+), in mice with cardiac-specific Dynamin-related protein 1 (Drp1) deletion (+/−/+), in mice with concomitant Parkin and Drp1 deletion (−/−/+) and in mice with Pink1 deletion (+/+/−). Right: mitophagy is reduced during ageing due to p53-dependent inhibition of Parkin.

suggests that mitophagy represents a fundamental mechanism for cardiac development[15] (Figure 3). Parkin deletion in *Drosophila* leads to dilated cardiomyopathy, due to the presence of abnormal mitochondria with disrupted or absent cristae.[51] Kubli and colleagues investigated cardiac function in systemic Parkin-knockout mice (Parkin −/−) and observed preserved cardiac function and mitochondrial function in unstressed conditions both in adult mice and in mice up to 12 months of age.[19] These findings suggest that Parkin is not critical for the turnover of mitochondria in the adult heart since other proteins may compensate for Parkin deficiency under baseline conditions. On the contrary, cardiac-specific inducible deletion of Parkin on perinatal day 1 is lethal in mice. Surviving mice display cardiac mitochondria with foetal features, likely due to the lack of a proper turnover of matured mitochondria.[52] In addition, inhibition of Parkin-mediated mitophagy through the overexpression of a mutant form of Mfn2 lacking Pink1 phosphorylation sites induces a lethal cardiomyopathy during perinatal life, but not after mouse weaning. Ultrastructural analyses in the hearts of these animas reveal failed maturation of mitochondria, which is paralleled by a functional switch of mitochondrial metabolism toward fatty acid utilization.[52] These results indicate that Parkin-mediated mitophagy is an essential mechanism during perinatal transition of cardiac metabolism, to allow a rapid replacement of mitochondria utilizing glucose with those utilizing fatty acids, which are indispensable to fuel increased contractile demand in adulthood. The preserved cardiac function observed in Parkin −/− mice also suggests that Parkin-independent mechanisms are instead required during prenatal cardiac development, which may compensate Parkin deficiency.

The role of Parkin-dependent mitophagy during cardiac development was also investigated in the presence of Drp1 deletion. Cardiomyocyte-specific Drp1 gene deletion in adult mice up-regulates Parkin expression and leads to the development of lethal dilated cardiomyopathy. Concomitant cardiac Parkin deletion in adult mice delays cardiac dysfunction caused by Drp1 deletion.[53] However, inducible deletion of Parkin during the adulthood or cardiomyocyte-specific Parkin overexpression does not affect cardiac function and morphology at baseline.[53] These results suggest that Parkin is dispensable for constitutive cardiac mitophagy but contributes to cardiomyopathy in the presence of fission inhibition, likely through mitochondrial depletion.

Another report showed that systemic Parkin deletion exacerbates cardiac dysfunction in a perinatal cardiac Drp1-deficient model.[54] Consistently, inducible cardiac Drp1 gene deletion during adulthood leads to left ventricular dysfunction and cardiac hypertrophy at baseline.[21] This model also displays reduced mitophagy and mitochondrial abnormalities, with the presence of fused mitochondria.

The role of Pink1 at baseline was also investigated. Systemic knockout of Pink1 leads to age-dependent cardiac hypertrophy and dysfunction starting from 2 months of age, along with mitochondrial dysfunction, reduced mitophagy and increased oxidative stress.[55] The latter indicates that Pink1 is crucial for postnatal cardiac development.

Tumour necrosis factor receptor-associated factor-2 (TRAF2), an innate immunity effector with E3 ubiquitin ligase activity, interacts with Parkin in depolarized mitochondria of neonatal rat cardiac myocytes and mediates cytoprotective TNF receptor signalling in the heart during stress.[56] Loss of TRAF2 impairs mitophagy at baseline in adult hearts and leads to cardiomyopathy, due to increased macrophage infiltration via toll-like receptor 9 (TLR-9) activation and cell death.[57] It would be interesting to evaluate whether mitophagy stimulation is able to rescue cardiac dysfunction in TRAF2-knockout mice. Moreover, the molecular mechanisms through which TRAF2 mediates mitophagy requires further investigations.

Stimulation of autophagy and mitophagy represents a potential anti-ageing strategy. During ageing, cardiac autophagy progressively declines, leading to the accumulation of dysfunctional and aged cellular elements, such as mitochondria.[58] In aged mice mitophagy is also reduced, due to the inhibition of Parkin by cytosolic p53. Parkin −/− mice show age-related cardiac dysfunction and decreased survival, whereas aged p53-deficient mice or mice with Parkin overexpression show less ageing-induced impairment of mitophagy and preserved cardiac function.[16] The ubiquitin-specific protease 30 (Usp30) was found to accelerate cell senescence in cardiac cells in association with reduced activity of Parkin and mitophagy. These effects are reversed by Parkin overexpression or Usp30 gene silencing.[59] In a recent work, impaired mitophagy was also observed in cardiomyocyte-specific RhoA conditional knockout (cKO) mice, along with increased cardiac ageing and age-dependent cardiac fibrosis.[60] RhoA gene knockout leads to the downregulation of Parkin expression and to the reduction of ubiquitinated mitochondrial proteins, while Parkin overexpression rescues cardiac dysfunction in RhoA cKO mice. Of interest, RhoA and Parkin expression were also reduced in heart samples of patients with severe heart failure caused by idiopathic dilated cardiomyopathy (DCM).[60] These results suggest that RhoA is an important regulator of Parkin-dependent mitophagy for the prevention of cardiac senescence and heart failure. Aged mice carrying a proofreading defective mtDNA polymerase γ (Polg) also display a reduction of Parkin-induced mitophagy. However, cardiac-specific overexpression of Parkin in mice expressing defective Polg does not rescue cardiac senescence, suggesting a minor role played by Parkin in clearing mitochondria with mtDNA damage during cardiac ageing.[61] Of interest, Liang et al. observed an increased number of mitochondria with specific molecular signatures allowing their recognition and subsequent elimination by the mitophagy process in the heart of aged mice, which was, however, associated with reduced expression of Atg9b and formation of autophagosome.[62] These data indicate that the aged heart is characterized by an unbalance between labelling and degradation steps of mitophagy.

3.2 | Cardiac hypertrophy and heart failure

Cardiac hypertrophy refers to the increase in the size of cardiomyocytes and can be caused by pressure or volume overload or by neuro-hormonal stimuli. After cardiac injury, such as infarction, the heart undergoes a series of morphological and molecular changes known as cardiac remodelling.[63] In the presence of an increase in cardiac afterload, cardiac hypertrophy acts as a compensatory response to reduce wall stress. However, in the long term, hypertrophy progresses toward cardiac dilation, systolic dysfunction and heart failure. Autophagy and mitophagy are activated in mice undergoing surgically-induced pressure overload (PO). One week after pressure overload (PO) induced by transverse aortic constriction (TAC), mice with cardiac-specific deletion of Atg5 develop cardiac dilation and dysfunction, associated with myocardial abnormalities, including mitochondrial defects.[64] In contrast, heterozygous Beclin 1 deletion improves cardiac function after a more severe form of PO, whereas Beclin1 overexpression increases pathological remodelling.[65] These results suggest that nonselective autophagy may exert both adaptive and maladaptive effects during PO. The preserved cardiac function observed in Beclin 1 knockout mice can be attributed to non-autophagy-related functions of Beclin 1. In this regard, caspase-mediated cleavage of Beclin-1 was found to inactivate Beclin-1 and to enhance apoptosis.[66,67]

Compared to nonselective autophagy, cardiac mitophagy appears to be activated only transiently during the acute phase of PO, from 3 to 7 days after TAC, being thereafter downregulated. The upregulation of mitophagy coincides with mitochondrial translocation of Drp-1, and mitochondrial dysfunction develops when mitophagy levels decrease. Cardiac-specific conditional Drp1 heterozygous knockout (Drp1-hetCKO) in mice accelerates mitochondrial dysfunction and heart failure, suggesting a pivotal role of Dpr1-dependent activation of mitophagy after PO.[17] The involvement of alternative forms of mitophagy that are independent of Atg5/7 and LC3 autophagosome conjugation system were also reported in mice undergoing TAC. Nah et al. found that mitophagy peaks at 1-day post TAC, through the conventional Atg7 pathway, while 3 days after TAC mitophagy is activated through Ulk1-dependent mechanisms. In fact, mice with cardiac-specific Ulk1 deletion develop a severe and early form of cardiac dysfunction in response to PO due to a defective Rab9-dependent alternative mitophagy. Restoration of mitophagy by Tat-Beclin 1, a potent inducer of autophagy, attenuates mitochondrial dysfunction and PO-induced heart failure.[18] This effect is also evident in Ulk1 and in Drp1 knockout mice, in which Tat-Beclin 1 administration reduces the progression of heart failure.[18] These results suggest that alternative mitophagy plays a pivotal role in the preservation of cardiac function during PO, compensating the earlier decline of conventional mitophagy[18] (Figure 4). Mitophagy inhibition in response to PO also represents a trigger for myocardial inflammation, another major player in the progression of cardiac remodelling. Otsu's group previously showed that during PO mitochondrial DNA escaping from autophagy-dependent elimination leads to cardiac dysfunction and heart failure by means of increased Toll-like receptor (TLR) 9-dependent inflammation.[68]

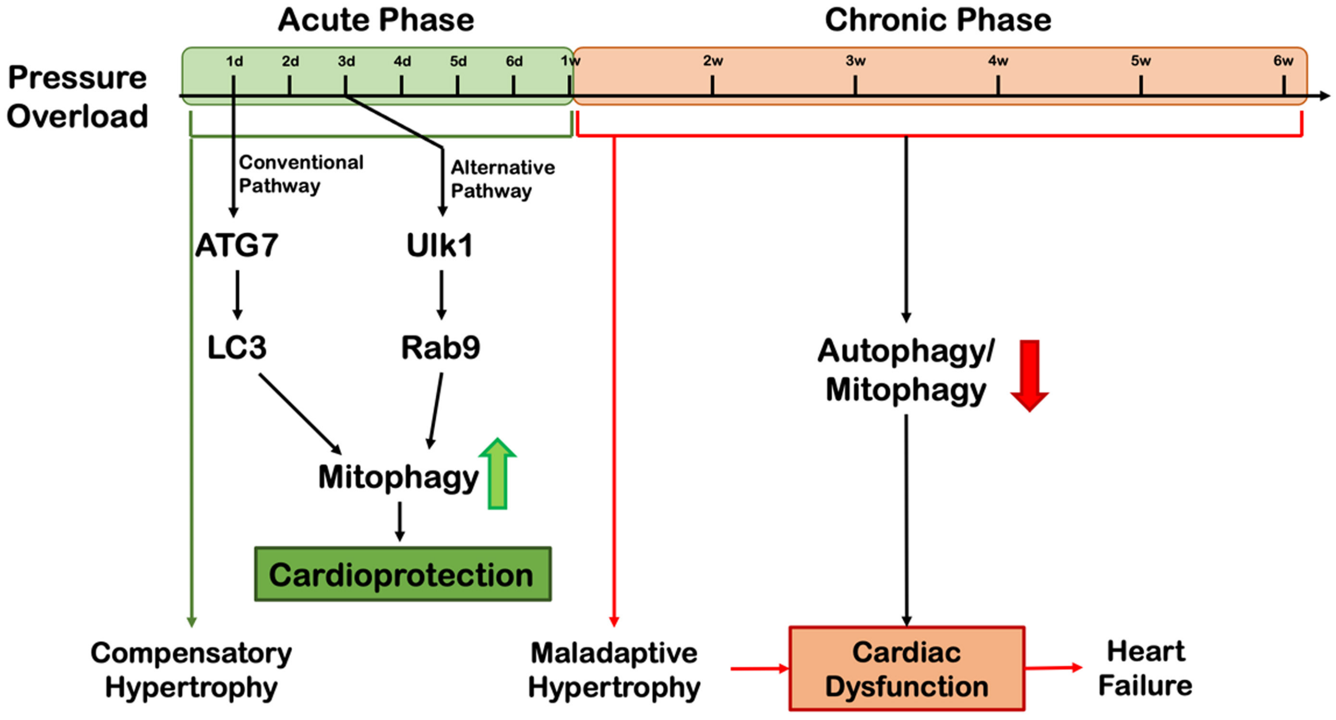


FIGURE 4 Role of mitophagy in response to pressure overload. Mitophagy displays a different pattern of activation compared to nonselective autophagy. Three days after pressure overload, the alternative mitophagy involving Ulk1 is activated. See text for further details. Atg7, autophagy related protein; LC3, microtubule-associated proteins 1A/1B light chain 3B; Rab9, Ras-related proteins; Ulk1, Unc-51 Like Autophagy Activating Kinase 1.

3.3 | Myocardial infarction and ischemia/reperfusion injury

The role of mitophagy was also studied in mouse models of myocardial infarction (MI) and ischemia/reperfusion (I/R) injury[19] (Figure 5). Generally, autophagy and mitophagy elicit beneficial effects in response to chronic MI.[11] Mice with systemic Parkin deletion undergoing permanent ligation of the left anterior descending coronary artery show reduced cardiac function and survival compared to wild-type mice. Of interest, in wild-type mice undergoing MI, Parkin is upregulated in the border zone of the infarct.[19] Overexpression of Parkin was also found to protect myocytes against hypoxia-induced cell death in vitro. These results suggest that Parkin-dependent activation of mitophagy represents an adaptive mechanism in response to MI, because it promotes the clearance of damaged mitochondria and ensures cardiac recovery after MI. However, Pink 1 was reported to be dispensable for Parkin recruitment to mitochondria in response to MI.[69] In fact, systemic Pink 1-knockout mice undergoing MI show increased Parkin recruitment to mitochondria, probably as a compensatory mechanism coping with Pink 1 deficiency. Further studies should characterize the mechanistic link between Pink 1 deficiency and the enhanced Parkin localization to mitochondria.

In the acute phase of MI, nonselective autophagy is generally activated during the ischemic phase, as an adaptive response that limits cardiac injury. In contrast, an excessive accumulation of autophagosomes in the heart was observed in response to I/R injury, partially due to flux inhibition in the later phase of reperfusion, and was reported to exacerbate I/R injury by triggering autosis, a form of cell death with peculiar morphological and biochemical characteristics that are distinct from apoptosis and necrosis.[70,71]

Increasing lines of evidence suggest that mitophagy exerts beneficial effects during acute ischemia and I/R injury. Saito et al. demonstrated that during ischemia, mitophagy is activated through the alternative pathway mediated by Ulk1/Rab9/Rip1 described above. In the same study, it was observed that over a 30-min period of myocardial ischemia, mitochondria engulfment by autophagosomes is impaired in the presence of cardiac-specific deletion of Ulk1. In addition, mice overexpressing a mutant form of Rab show exacerbated injury in response to 2h of myocardial ischemia.[20] These results suggest that alternative mitophagy confers cardioprotection by improving mitochondrial function and that mitophagy and nonselective autophagy are regulated by different mechanisms in response to ischemia.

Regarding the role of mitophagy in models of I/R injury, it was reported that the expression of Drp1

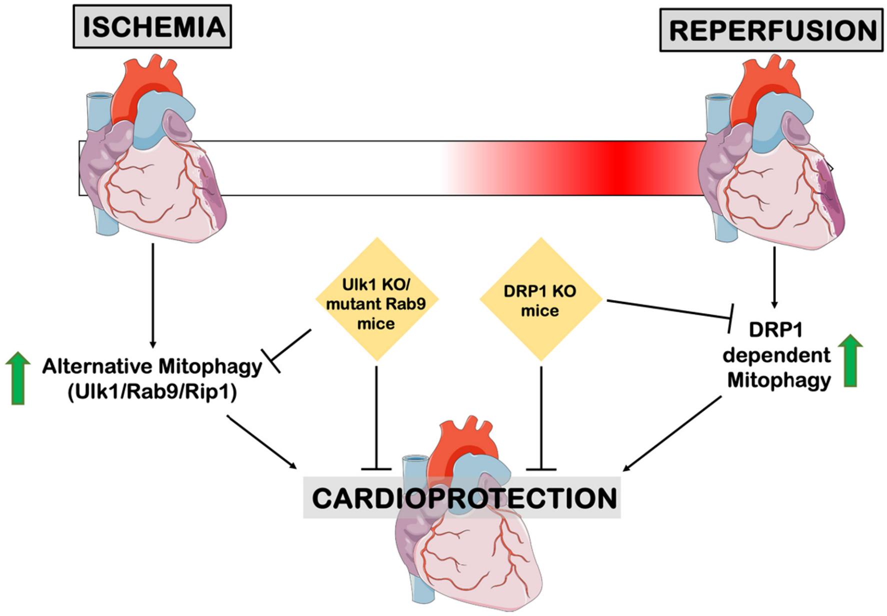


FIGURE 5 Role of mitophagy in response to ischemia and reperfusion. Mitophagy is activated in response to ischemia and reperfusion, by alternative mechanisms and DRP-1, respectively. Mice with genetic inhibition of ULK1, Rab9 or Drp-1 show an increased cardiac injury in response to ischemia and reperfusion, respectively. Legend: DRP1, Dynamin-related protein 1; Ulk1, Unc-51 Like Autophagy Activating Kinase 1; Rab9, Ras-related proteins; Rip1, receptor-interacting serine/threonine protein kinase 1. Elements were modified from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic Licence. https://smart.servier.com/; https://creativecommons.org/licenses/by/3.0/

increases and cardiac-specific Drp1 knockout mice show increased infarct size in response to I/R injury, together with the accumulation of dysfunctional mitochondria due to reduced mitophagy.[21] Furthermore, mice lacking the phosphoglycerate mutase family member 5 (Pgam5), a mitochondrial protein that is associated with Rip1/Rip3, develop increased necroptosis along with reduced Pink1-dependent mitophagy.[22] Treatment with bicarbonate, as a mimetic of oxygen consumption, increases I/R injury and impairs clearance of mitochondria.[72] Boosting mitophagy by simvastatin decreases I/R injury in mice, through a Parkin-dependent mechanism.[73] In a recent study, mitophagy was reported to be regulated by the circadian core regulatory gene Clock during acute MI. Mice carrying a mutant form of Clock undergoing I/R show increased cardiac dysfunction and impaired mitochondrial turnover. Conversely, the observed beneficial effects of Clock on cell viability are abrogated by autophagy inhibition.[23]

Ischemic preconditioning (IPC), which refers to brief episodes of myocardial ischemia before a subsequent prolonged ischemic, represents an attractive intervention to reduce I/R injury. Mitophagy was reported to mediate the myocardial protective effects of IPC in mice. In fact, the cardioprotective effects of IPC are blunted in Parkin −/− mice.[24] Another study found that hypoxic preconditioning reduces I/R injury in mice through the activation of mitophagy in platelets, in a mechanism mediated by Fundc-1.[74] These results suggest that platelet mitophagy is an important contributor of the protective effects of hypoxic preconditioning. Future studies should test whether a selective activation of mitophagy at an early time point after reperfusion is also able to reduce I/R injury, mimicking postconditioning.

3.4 | Metabolic cardiomyopathy

Metabolic alterations induced by diabetes, obesity or metabolic syndrome represent leading causes of cardiomyopathy. Adult mice fed a high-fat diet (HFD) for 18–20 weeks to induce obesity and metabolic syndrome show inhibition of autophagy and increased cardiac injury when subjected to prolonged ischemia, through uncontrolled mTORC1 activation. Pharmacological mTORC1 inhibition reduces ischemic injury in HFD-treated mice.[75] The BCL2 AAA mouse, an animal model unresponsive to autophagy stimulation, due to a mutation in BCL-2 which inhibits the dissociation of the BCL2–Beclin-1 complex, was not found to be protected from HFD-induced glucose intolerance.[76] The administration of rapamycin, an mTORC1 inhibitor, rescues cardiac hypertrophy and contractile dysfunction in HFD-treated mice.[77] At the molecular level, in addition to mTORC1 activation, Xie et al. observed a decreased cardiac activity of AMPK in OVE26 diabetic

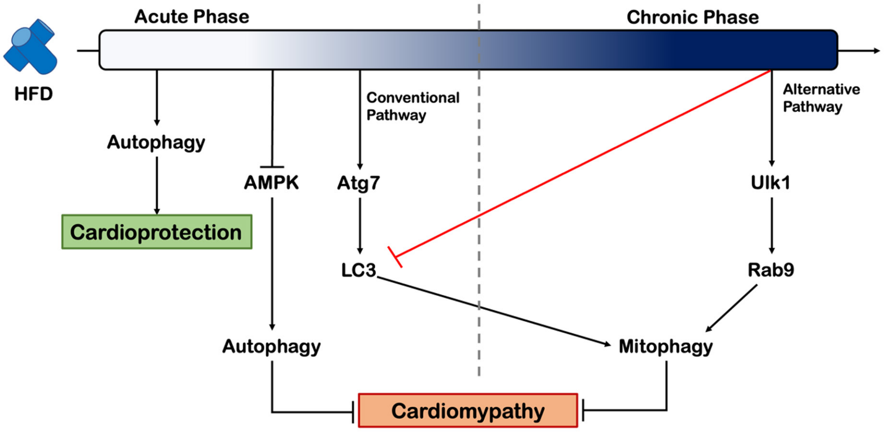


FIGURE 6 Role of mitophagy during high-fat diet (HFD)-induced cardiomyopathy. During the acute phase of HFD consumption, mitophagy is active through a conventional pathway, mediated by Atg7. Mitophagy remains active during chronic HFD consumption, through the Ulk1/Rab9 pathway. See text for further details. AMPK, activated mitogen protein kinase; Atg7, autophagy related gene 7; LC3, microtubule-associated proteins 1A/1B light chain 3B; Ulk1, Unc-51 Like Autophagy Activating Kinase 1; Rab9, Ras-related proteins.

mice and demonstrated that metformin, an AMPK activator, reduces diabetes-induced cardiomyopathy.[78] Interestingly, in another study performed in Type 1 diabetic mice, cardiac dysfunction was attenuated by genetic inhibition of autophagy, achieved by Beclin 1 or Atg16 deletion.[79] Genetic inhibition of autophagy during Type 1 diabetes leads to mitophagy activation, suggesting that the diminished autophagy is an adaptive response which limits diabetes-induced cardiac injury.[79] The molecular mechanisms through which autophagy inhibition is associated with restoration of mitophagy were not studied in this work. One possible explanation is the involvement of alternative mitophagy, since cardiac expression of Rab9 increases in response to diabetes.[79]

Mitophagy was shown to play a fundamental role in HFD-treated mice (Figure 6). Cardiac autophagic flux is upregulated in the early phase of HFD consumption and inhibited in a late phase. In contrast, mitophagy level peaks after 2 months of HFD. Cardiac deletion of Atg7 or Parkin inhibits mitophagy and aggravates cardiac dysfunction in mice fed a HFD for a short period of time, with a mild phenotype observed in Parkin −/− animals. Mitophagy reactivation through Tat-Beclin 1 reduces mitochondrial dysfunction, lipid accumulation and cardiac dysfunction in HFD wild-type mice,[25] indicating that although activated in early phase of HFD, autophagy-dependent elimination of mitochondria is not sufficient to prevent mitochondrial dysfunction. In addition, these results suggest that mitophagy serves as protective mechanism in response to HFD through an Atg7-dependent mechanism in the early phase of HFD, with a partial involvement of Parkin. In fact, cardiac expression of Parkin decreases in obese mice after 12 weeks of HFD consumption[80] suggesting that the increase in mitophagy observed despite the reduction in Parkin involves alternative forms of mitophagy.

In this regard, in a subsequent study, Tong et al. investigated the molecular mechanisms that mediate mitophagy activation during chronic HFD administration. Mitophagy remains elevated after 24 weeks of HFD feeding. At this time point, conventional Atg5/7 autophagy is decreased compared with littermate controls, with significant inhibition of LC3 levels in total and mitochondrial lysates. On the other hand, Ulk1 phosphorylation at Ser555 increases, leading to Rab9 recruitment to mitochondria. Cardiac deletion of Ulk1 or selective inhibition of alternative mitophagy through a mutant Rab9 (Rab9 S179A knock-in) exacerbates cardiac dysfunction in response to HFD. Stimulation of alternative mitophagy through cardiac overexpression of Rab9 rescues cardiac dysfunction in response to chronic administration of HFD. Mechanistically, transcription factor binding to IGHM enhancer 3 (Tfe3), a transcriptional regulator of autophagy and lysosomal biogenesis is upregulated in the heart after 12 weeks of HFD. Of interest, HFD does not induce Rab9 upregulation and alternative mitophagy in mice with cardiac-specific Tfe3 knockout. This indicates that Tfe3 is involved in the transcriptional regulation of alternative mitophagy during HFD consumption.[26,81] In a recent work, Drp1 was reported to play a fundamental role in mediating both conventional and alternative mitophagy during HFD consumption in mice. In fact, conventional and alternative mitophagy were abolished in tamoxifen-inducible cardiac-specific Drp1 knockout mice fed with HFD. Drp1 is phosphorylated at Ser616 during the chronic phase of HFD and results to be colocalized with Rab9 and Fis1 in the mitochondria-associated membranes. These data suggest that Drp1 is involved in alternative mitophagy during the chronic phase of HFD consumption.[27]

4 | MITOPHAGY AND VASCULAR SYSTEM

4.1 | Mitophagy and atherosclerosis

Atherosclerosis is a multifactorial condition which represents the pathophysiological substrate of most of CVDs and encompasses a series of events that involve platelet and endothelial activation, vascular smooth muscle cells (VSMCs) proliferation and immune cells recruitment.[58,82] A large body of evidence suggests that autophagy and mitophagy play a fundamental role in the vascular system at baseline and in response to stress, although the molecular mechanisms involved are less characterized compared to the heart.

4.1.1 | Macrophage mitophagy and atherosclerosis

Autophagy is impaired in mouse models of atherosclerosis and in patient specimens, with a marked accumulation of autophagic markers reported in macrophages.[83,84] Specific deletion of Atg5 in macrophages increases plaque formation, along with inflammation.[83,84] Autophagy is also suppressed in human macrophages in vitro in response to growth differentiation factor-15 (GDF-15) and oxidized low-density lipoproteins (oxLDL).[85] In line with this evidence, restoration of autophagy in macrophage through transcription factor EB (Tfeb) overexpression or by trehalose, a natural activator of autophagy and inducer of Tfeb, was found to reduce atherosclerosis in mice.[86] Inhibition of autophagy in fat-fed low-density lipoprotein (LDL) receptor (Ldlr) knockout mice (*Ldlr* −/−), exacerbates oxidative stress and promotes plaque necrosis in advanced atherosclerosis.[87] These results suggest that macrophage autophagy plays a protective role during atherosclerosis.

Few studies investigated the role of mitophagy in macrophages during atherosclerosis. In a recent paper, in mice with Apolipoprotein A-I binding protein (Aibp) deletion (Aibp −/−), macrophage autophagy was found to be downregulated in atherosclerotic lesions, along with increased apoptosis. Aibp interacts with Parkin and mitophagy is reduced in Aibp −/− macrophages exposed to OxLDL in vitro.[88] These data indicate that Parkin-dependent mitophagy may act as an adaptive mechanism in response to stress.

mTORC1 has also emerged as a regulator of macrophage mitophagy during atherosclerosis. Atherosclerosis is exacerbated in apolipoprotein E-deficient (Apo E −/−) mice fed a high-protein diet along with mTOR activation in plaque macrophages. The latter was associated with mitophagy suppression. Inhibition of mTOR through macrophage-specific deletion of Raptor, a fundamental component of mTORC1, abrogates the deleterious effects of high-protein diet on atherosclerosis progression.[40] In HFD-fed Apo E −/− mice, the endoplasmic reticulum (ER) stress inhibits Parkin-mediated mitophagy by activating the integrated stress response (ISR).[89]

4.1.2 | VSMCs mitophagy and atherosclerosis

The importance of autophagy and mitophagy in VSMCs during atherosclerosis has also emerged in recent years. VSMC-specific Atg7 knockout mice display increased neointima formation after ligation of the left common carotid artery and enhanced atherogenesis and vascular senescence in response to western diet administration.[90] These results were also confirmed in another study performed in ApoE −/− mice carrying VSMC-specific deletion of Atg7. In response to a western diet, impairment of autophagy was associated with maladaptive arterial remodelling, aortic rupture and cell death.[91] The impairment of mitophagic flux was also observed, along with the presence of fragmented mitochondria and oxidative stress in atherosclerotic plaques.[91,92] In human VSMCs exposed to oxLDL in vitro, Pink1-Parkin-dependent mitophagy is upregulated, and its forced overexpression rescues LDL-induced apoptosis.[93] These results suggest that mitophagy activation in VSMCs in response to atherogenic stressors acts as an adaptive mechanism devoted to stabilization of atherosclerotic plaque.[93] Apelin, the endogenous ligand of APJ receptor, a receptor that resembles the angiotensin II type 1 receptor, was reported to increase VSMC proliferation and atherosclerotic lesions in Apo E −/− mice, in association with an increase of mitophagy. These effects were abrogated in mice with genetic inhibition of PINK1.[94] This study suggests that excessive activation of mitophagy, for example during apelin treatment, may lead to maladaptive effects during atherosclerosis.

4.1.3 | Endothelial cell mitophagy and atherosclerosis

The involvement of endothelial autophagy and mitophagy in the pathogenesis of atherosclerosis was also assessed. Shear forces generated by blood flow in arteries stimulate endothelial autophagic flux.[95] Endothelial deficiency of Atg5 in Apo E −/− mice promotes atherosclerosis and increases apoptosis, inflammation and senescence.[95,96] Additional evidence also demonstrated that endothelial cells treated with oxLDL show an impairment of mitophagy and mitochondrial fusion, which in turn contributes to enhanced apoptosis and oxidative stress.[97] Restoration of mitophagy in endothelial cells in vitro in response to metabolic stress improves viability, mitochondrial function and reduces oxidative stress.[98] In addition, Wu et al. observed an increased expression of Pink1 and Parkin in vascular wall and endothelial cells of obese and diabetic mice.[99] These results suggest that endothelial mitophagy is activated in response to stress, exerting adaptive effects.

Sirt-3 has emerged as a potential regulator of Parkin-dependent mitophagy in endothelial cells. Sirt-3 interacts with Pink1 and Parkin and induces their deacetylation, which in turn contributes to the promotion of mitophagy. In fact, Sirt3 overexpression in cardiac microvascular endothelial cells enhances Pink1/Parkin-induced mitophagy and Sirt3 deficiency leads to impaired angiogenesis in hypertensive mice, also suppressing mitophagy.[100] Future studies should test whether the beneficial effects of Sirt3 overexpression in endothelial cells are attenuated in the presence of Pink1/Parkin deficiency.

4.1.4 | Platelet mitophagy in atherosclerosis

Platelet hyperaggregation is a pathophysiological contributor to thrombosis. Platelet isolated from subjects at high risk of developing CVDs, such as smokers, patients with atrial fibrillation and with metabolic syndrome show reduced autophagy, which correlates with increased platelet activation and oxidative stress.[101]

Among risk factor, diabetes is a major cause of atherosclerosis and atherothrombosis. Levels of markers of mitophagy, such as Pink-1 and Parkin, are higher in platelets isolated from diabetic patients, as adaptive response to reduce oxidative stress-induced apoptosis. In fact, autophagy inhibition increases apoptosis whereas autophagy stimulation reduces platelet aggregation in diabetic platelet ex-vivo, due to the removal of dysfunctional mitochondria.[102] In line with this evidence, lack of mitophagy, through Pink1 deletion, increases platelet activation and thrombosis progression in mice.[102] These results suggest that mitophagy exerts adaptive mechanisms in platelets in response to diabetes.

4.2 | Vascular ageing and mitophagy

Vascular ageing represents a risk factor for the development of atherosclerosis. Tyrrell et al. reported that ageing increases the levels of pro-inflammatory cytokine interleukin-6 (IL-6) in aorta of mice, and this was associated with increased Parkin-mediated mitophagy and mitochondrial dysfunction. Aortic levels of nonselective autophagy were not affected by ageing in aorta. Hyperlipidaemia further increases mitophagy in aged aorta, accelerating atherosclerosis. Surprisingly, pharmacological activation of mitophagy by spermidine in aged hyperlipidaemic mice reduces IL-6 and Parkin levels and improves mitochondrial function, which in turn reduces atherosclerosis.[103] These results suggest that the increase of mitophagy observed during ageing is paralleled by the accumulation of dysfunctional mitochondria. However, another study found that the reduced levels of markers of nonselective autophagy in endothelial cells isolated from the brachial artery of older adults correlates with the impairment of endothelium-dependent dilatation.[104]

4.3 | Mitophagy and stroke

Stroke may represent a consequence of vascular dysfunction, due to hypertension, genetic and lifestyle factors. In a hypertensive rat model of spontaneous stroke, autophagy and mitophagy resulted impaired in the brain and in isolated cerebral endothelial cells, because of mitochondrial dysfunction. Reactivation of autophagy/mitophagy through specific compounds, such as nicotinamide mononucleotide, trehalose and Tat-Beclin D11 rescues mitochondrial dysfunction and reduces endothelial dysfunction and stroke occurrence.[105,106]

5 | MITOPHAGY AS THERAPEUTIC TARGET IN CARDIOVASCULAR DISEASES

We reviewed recent data indicating that restoration of mitophagy improves cardiac remodelling and reduces ischemic injury and cardiac complications related to metabolic disorders. Emerging evidence also supports a potential role of mitophagy activation in delaying vascular ageing and atherosclerosis. Different studies also suggested that mitophagy components are differentially expressed in cardiac samples of patients with cardiac diseases compared with control subjects.[107,108] For example, Andres et al. reported that mitophagy is activated in atrial tissue of patients undergoing heart surgery with cardiopulmonary bypass.[108] Mitochondrial alterations together with a reduced expression of mitophagy genes, such as Bnip3, Fundc1, were detected by RNA-seq analysis in myocardial samples of patients with hypertrophic cardiomyopathy (HCM) and compared to control samples from donor hearts.[107] Another study demonstrated a decreased expression of markers of mitophagy (i.e. PINK1, PARKIN, PARL, FUNDC1) and autophagy (LC3, Beclin 1) in myocardial samples from patients with ischemic and dilated cardiomyopathy in a terminal stage of heart failure.[109] However, the increase of markers of autophagy (LC3, Beclin 1, ATG5-12, p62) and mitophagy (Parkin) at early reperfusion times was not observed in left ventricle myocardial biopsies of patients undergoing coronary artery bypass grafting (CABG) and subjected to remote ischemic preconditioning (RIPC).[110]

A different pattern of expression of autophagy genes, such as MAP1LC3B, RAB24, and EVA1A was found between carotid plaques of patients with unstable plaques compared to asymptomatic patients.[111] Microdissection of human carotid plaques from patients undergoing carotid atherectomy displays an increased expression of Pink1 mRNA within plaque cap, along with a metabolic switch from oxidative phosphorylation to glycolysis.[112]

Mitochondrial dysfunction, due to increased oxidative stress and altered mitochondrial dynamics, including mitophagy, is the pathological substrate of several CVDs. Thus, restoration of these process may be a promising therapeutic strategy for clinicians.

To date, different strategies have been developed to improve mitochondrial function. In most cases, the reduction of mitochondrial oxidative stress has emerged as the most efficacious. Mitochondrial-targeted antioxidants are being tested in ongoing clinical trials. For example, the mitochondrial antioxidant MitoQ was reported to improve vascular function in healthy older adults.[113] Restoration of NAD represents another suitable approach to rescue mitochondrial dysfunction in models of CVDs and to decrease the risk of heart failure and cardiac mortality in patients.[114–116] However, it has not been established yet whether administration of these compounds normalizes cardiovascular mitophagy during the disease course.

Known activators of autophagy, such as metformin and rapamycin, exert their beneficial effects on health through off-target mechanisms in addition to autophagy, making difficult their application as specific activators of mitophagy.[42] In contrast, spermidine and trehalose, two natural compounds, were reported to induce both nonselective autophagy and mitophagy in cardiomyocytes in vitro and in preclinical models of CVDs.[117,118] Spermidine, a natural polyamine, increases autophagy by the inhibition of EP300, a known inhibitor of autophagy. Spermidine was reported to improve longevity in mice and to exert cardioprotective effects through autophagy and mitophagy.[38,117] Trehalose is a natural disaccharide which stimulates autophagy and mitophagy in the heart by increasing the activity of TFEB, as discussed before[118] (Figure 7). Recent clinical studies demonstrated that a combination of natural activators of autophagy, including trehalose and spermidine, reduces oxidative stress, inflammation and endothelial dysfunction in patients with peripheral vasculopathy and hypertension.[119,120] Ongoing clinical studies are also recruiting participants to test the effects of spermidine on hypertension, in patients with heart failure with preserved ejection fraction and in elderly patients with chronic ischemic heart disease (NCT04405388; NCT05128331; NCT06186102). If the beneficial effects of spermidine will be confirmed in these studies, experimental evidence may be rapidly transferred to the clinical arena. However, additional trials are required to test the effects of autophagy activators in large population and in different cohorts, such as patients with heart failure with reduced ejection fraction or with acute coronary syndrome. Whether a combination of different modulators of autophagy would provide greater benefits than the administration of a single compound should also be confirmed in large population studies. We discussed that the synthetic compound Tat-Beclin 1, which activates autophagy by competing with the negative regulator of Beclin 1 GLI pathogenesis related 2 (Gapr-1/Glipr-2) on the Golgi surface, also improves cardiac mitophagy.[17] To date, no clinical studies are reported the use of Tat-Beclin 1. To the best of our knowledge, specific compounds able to target mitophagy without affecting nonselective autophagy have not yet been characterized.

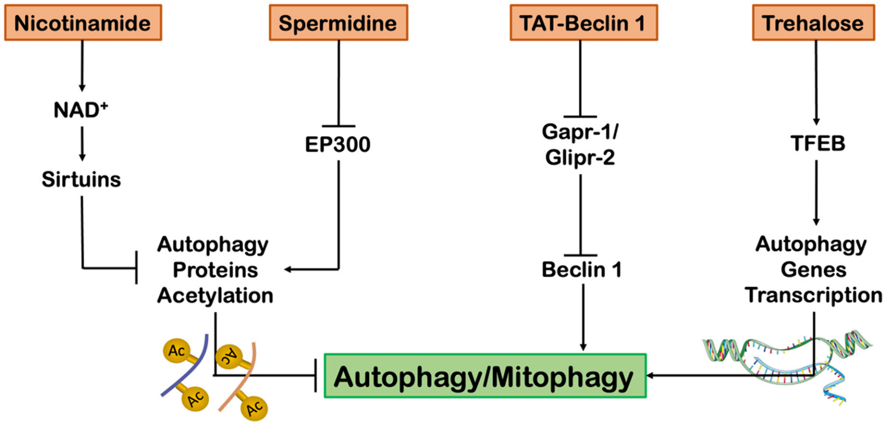


FIGURE 7 Mitophagy as therapeutic target for cardiovascular diseases. Main compounds able to enhance cardiac autophagy and mitophagy and their mechanisms of action. See text for further details. Elements were modified from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic Licence. https://smart.servier.com/; https://creativecommons.org/licenses/by/3.0/

6 | CONCLUSIONS AND PERSPECTIVES

We reviewed current literature about the fundamental importance of mitophagy in cardiac and vascular physiology and its translational relevance for human disease. Although increasing lines of evidence converge towards the concept that mitophagy activation exerts beneficial effects in CVDs and reduces mitochondrial dysfunction, some aspects related to these processes still require further investigations. We highlighted that the activation of nonselective autophagy in some circumstances may be detrimental, since it may activate cell death mechanisms, such as autosis, as it may occur for example during late reperfusion injury. On the other hand, mitophagy appears to reduce I/R damage. For this reason, the characterization of compounds capable of targeting mitophagy without affecting nonselective autophagy are encouraged. In this case, an interesting approach may be the development of molecules able to target specific markers of alternative forms of mitophagy, such as Rab9, which act independently of the molecular mediators involved in conventional autophagy. Furthermore, the time window during which activation of mitophagy would give the greatest benefits should be further characterized, as preventive or therapeutic strategy in patients at high risk or in patients with overt disease, respectively. Although the consensus is that mitophagy plays a protective role in the heart in response to various stress, few evidence also suggests that excessive levels of mitophagy may lead to detrimental effects, as observed in a mouse model of doxorubicin-induced cardiotoxicity.[121] Another major issue is that the molecular mechanisms by which mitophagy declines after a rapid increase in the stressed heart are not fully understood. The latter should be addressed by studying the involvement of known regulators of nonselective autophagy, such as AMPK, mTOR, TFEB. It is also not clear the role of mitophagy in other cardiac cells, such as fibroblast. In this regard, mitophagy downregulation in cardiac fibroblast was reported to reduce cardiac fibrosis.[122] A fully characterization of molecular mechanisms involved in the regulation of mitophagy in cardiac resident immune cells also requires further investigations. A deeper understanding regarding the molecular mechanisms underlying mitophagy activation during atherosclerosis in different cell types also represent a future challenge. While the effects of natural autophagy/mitophagy activators such as trehalose and spermidine are being tested in patients, diagnosis of mitophagy in the human heart and vessel samples still represents a big concern. It would be interesting to identify circulating biomarkers of mitophagy allowing risk stratification in subjects at high risk of developing CVD. In this regard, a recent study performed in patients with thoracic aortic aneurysm highlights the possibility to detect markers of nonselective autophagy, such as p62 and ATG5, in the plasma, also demonstrating a linear correlation with aortic tissue levels of markers of autophagy.[123] Further studies should validate these findings in larger populations with different CVDs and correlation analyses should be corroborated by cause-and-effect studies. Another interesting approach for the diagnosis of mitophagy would be the identification of predictive/prognostic single nucleotide polymorphisms (SNPs) in mitophagy genes associated with CVDs, to develop new polygenetic risk scores for these patients and to improve therapy. To date, several SNPs in the autophagy related (ATG) genes were demonstrated to be associated with several complex diseases,[124] but evidence regarding CVD is still scarce. The clarification of this important aspect may help to get new insights into the role of mitophagy in patients and to improve medicine and risk stratification. Finally, it will be important to better understand the potential side effects of a pharmacological activation of autophagy in subjects with CVD, preferring natural activators and carefully selecting the appropriate therapeutic window and clinical context of application.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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