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Mitophagy: At the heart of mitochondrial quality control in cardiac aging and frailty

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ABSTRACT

Cardiovascular disease is highly prevalent among older adults and poses a huge burden on morbidity, disability, and mortality. The age-related increased vulnerability of the cardiovascular system towards stressors is a pathophysiological trait of cardiovascular disease. This has been associated with a progressive deterioration of blood vessels and decline in heart function during aging. Cardiomyocytes rely mostly on oxidative metabolism for deploying their activities and mitochondrial metabolism is crucial to this purpose. Dysmorphic, inefficient, and oxidant-producing mitochondria have been identified in aged cardiomyocytes in association with cardiac structural and functional alterations. These aberrant organelles are thought to arise from inefficient mitochondrial quality control, which has therefore been place in the spotlight as a relevant mechanism of cardiac aging. As a result of alterations in mitochondrial quality control and redox dyshomeostasis, mitochondrial damage accumulates and contributes to cardiac frailty. Herein, we discuss the contribution of defective mitochondrial quality control pathways to cardiac frailty. Emerging findings pointing towards the exploitation of these pathways as therapeutic targets against cardiac aging and cardiovascular disease will also be illustrated.

1. Introduction

Cardiovascular disease (CVD) poses a huge morbidity, disability and mortality burden on the general population and is highly prevalent among older adults (Virani et al., 2020). People aged 80 years and older are at higher risk of heart failure, atrial fibrillation, and related stroke (Virani et al., 2020). Age-related increased vulnerability of the cardiovascular system towards stressors has been associated with progressive deterioration of blood vessels and decline in heart function (Chiao and Rabinovitch, 2015). In particular, an increase in heart mass, ventricular wall thickness, and cardiomyocyte cross-sectional area have been indicated as phenotypical manifestations of cardiac aging (Tracy et al., 2020).

Cardiomyocytes rely mostly on oxidative metabolism for deploying their activities and mitochondria are crucial organelles for cardiac functioning by supplying energy for myocardial contraction (Bertero and Maack, 2018; Murphy et al., 2016). Cardiac tissue is enriched in mitochondria that account for about 30% of myocellular volume with the ability of using several metabolic substrates to generate ATP under a wide range of physiological and pathological conditions (Bertero and Maack, 2018; Murphy et al., 2016). Along with their role of the cell's powerhouse, mitochondria are also a hub of several other activities

Abbreviations: AMPK, 5′-AMP-activated protein kinase; AMBRA1, autophagy and beclin-1 regulator 1; ANT, adenine nucleotide translocator; ATG, autophagy-related protein; CVD, cardiovascular disease; BCL2, B-cell lymphoma 2; BNIP3, BCL-2 interacting protein 3; DISC1, disrupted-in-schizophrenia-1; ER, endoplasmic reticulum; ETC, electron transport chain; EV, extracellular vesicle; FIP200, focal adhesion kinase family interacting protein of 200 kDa; FUNDC1, mitochondrial receptor FUN14 Domain Containing 1; IGF1, insulin like growth factor 1; JNK, c-Jun N-terminal kinase; ILVs, intraluminal vesicles; I-R, ischemia-reperfusion; LC3, microtubule-associated proteins 1A/1B light chain 3B; MDV, mitochondrial derived vesicle; MQC, mitochondrial quality control; Mst1, mammalian Ste20-like kinase 1; mtDNA, mitochondrial DNA; mTORC, mechanistic target of rapamycin complex 1; MVBs, multivesicular bodies; NDP52, nuclear domain 10 protein 52; OMM, outer mitochondrial membrane; OPTN, optineurin; OXPHOS, oxidative phosphorylation; PARL, presenilin-associated rhomboid-like protein; PINK1, phosphatase and tensin homolog-induced kinase 1; RHEB, GTP-binding protein Ras homolog enriched in brain; ROS, reactive oxygen species; TIM23, translocase of inner mitochondrial membrane 23; TOM, translocase of the outer mitochondrial membrane; ULK1, Unc-51-like kinase 1; VPS, vacuolar protein sorting.

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including the regulation of metabolic reactions, cell death, calcium storage, and reactive oxygen species (ROS) production (Picca et al., 2021).

Dysmorphic and inefficient, high ROS-producing mitochondria have been described in aged cardiomyocytes (Dutta et al., 2012) together with cardiac structural and functional alterations (Marzetti et al., 2009). Therefore, mitochondrial dysfunction and inefficient mitochondrial quality control (MQC) processes have been placed in the spotlight as factors in cardiac aging (Picca et al., 2018a). Defective MQC and the installment of oxidative stress in the heart may be envisioned as an outcome of unsuccessful aging rather than a phenotypic expression of aging itself (Inglés et al., 2014). Indeed, altered quality control signaling and imbalanced oxidant defense may contribute to cardiac frailty as a result of damage accumulation not fully compensated by resilience mechanisms. When approaching the late stages of life, resiliency may become overwhelmed and stressors may cause rapid and unopposed damage accumulation that leads to frailty and eventually death. Accelerated aging may ensue because of either faster rates of damage accumulation or rapid shrinking and eventual collapse of resilience (Ferrucci et al., 2020). In this setting, peculiar cardiac ultrastructural changes have also been observed and associated with physical frailty (Pelà et al., 2021b, 2021a).

Physical activity and exercise are recognized strategies and highly recommended interventions to prevent and manage CVD (Arnett et al., 2019; Haskell et al., 2007). Several observational studies have shown that the lack of compliance with physical activity recommendations is associated with an increased risk of myocardial infarction, coronary heart disease, stroke, and death (Blair et al., 1995; Chomistek et al., 2013; Held et al., 2012; Talbot et al., 2007). The effects of physical exercise on cardiovascular health go beyond prevention and also include significant changes in cardiac structure and function in the presence of CVD (Abad et al., 2017; C. Moraes-Silva et al., 2017; Feriani et al., 2018). Although many potential mechanisms have been suggested to explain such beneficial effects, improvements in mitochondrial function following physical exercise have received special attention (Guan et al., 2019).

Here, we discuss mitophagy and the generation of mitochondrial derived vesicles (MDVs) as relevant pathways in MQC and their involvement in cardiac frailty. The possibility of targeting MQC pathways to obtain therapeutic gain against cardiac aging is also discussed.

2. Autophagy and mitophagy in cardiomyocytes

As an organ virtually postmitotic, the heart is among the most robust autophagy recipients of the body and relies on this degradative route for maintaining homeostasis (Sun et al., 2015). In keeping with this is the observation that upregulation of autophagy and mitophagy occurs following ischemia-reperfusion (I-R) and sepsis (Hoshino et al., 2012). Furthermore, an attenuation of stress-induced mitochondrial autophagy, accompanied by altered mitochondrial function and impaired cardiac function, has been observed in mouse models lacking the mitophagic regulator Parkin (Hoshino et al., 2012; Kanamori et al., 2011a, 2011b; Piquereau et al., 2013).

The cardiomyocyte energy balance modulates cardiac autophagy via metabolic signaling. A drop in cardiomyocyte ATP in case of substrate deficiency or oxidative stress triggers the activation of a pro-autophagy pathway involving 5′-AMP-activated protein kinase (AMPK), unc-51-like kinase 1 (ULK1), B-cell lymphoma 2 (BCL2), and Beclin-1 signaling (Egan et al., 2011; Maejima et al., 2016). A concomitant downregulation of the autophagy suppressor mechanistic target of rapamycin complex 1 (mTORC1) further supports autophagy-mediated degradation (Tan and Miyamoto, 2016). Anti-autophagic stimuli may be conveyed via insulin/insulin-like growth factor 1 (IGF1)/protein kinase B signaling to circumvent the possibility of excessive autophagy and, thus, hyper-degradation (Ock et al., 2016). As a reinforcing anti-autophagic action, the GTP-binding protein Ras homolog enriched in brain (RHEB) quenches autophagy via mTORC1 signaling and transcription factors related to lysosomal biogenesis (Sciarretta et al., 2012).

A selective form of autophagy, referred to as mitophagy, is in charge of degrading dysfunctional and depolarized mitochondria in order to preserve mitochondrial efficiency in cardiomyocytes. This process occurs in coordination with a set of other quality control mechanisms, including mitochondrial biogenesis, dynamics, and proteostasis (Picca et al., 2018a). Mitophagy operates via phosphatase and tensin homolog-induced kinase 1 (PINK1)-Parkin-dependent and independent pathways (Fan et al., 2020) (Fig. 1).

PINK1-Parkin-dependent degradation has been involved in preserving mitochondrial and cardiac function in diabetic mice with cardiomyopathy induced by a high-fat diet regimen (Tong et al., 2019). Conversely, defective PINK1-Parkin-dependent mitophagy has been implicated in the severe cardiac complications observed in animal models of Duchenne muscular dystrophy (Fan et al., 2020). The preservation of cardiac function via the promotion of Parkin-dependent mitophagy has also been reported in a model of septic cardiomyopathy (Shang et al., 2020). In this context, the benefit conveyed by the activation of the mitophagy route seems to be achieved via the downregulation of the expression of the negative regulator of cardiomyocyte mitophagy, mammalian Ste20-like kinase 1 (Mst1), which is able to attenuate lipopolysaccharide-induced cardiomyocyte death (Shang et al., 2020). Finally, a role for PINK1-driven mitophagy has also been reported via the interaction of the adenine nucleotide translocator (ANT) complex with the translocase of inner mitochondrial membrane 23 (TIM23) (Hoshino et al., 2019). Dysmorphic mitochondria associated with cardiomyocyte hypertrophy and contractile dysfunction have been identified in the murine heart depleted of ANT (Hoshino et al., 2019). Of note, homozygous mutations of ANT1 in humans have been associated with severe heart failure and cardiac mitochondrial dyshomeostasis (Hoshino et al., 2019).

The PINK1-Parkin-independent mitophagy pathway operates via inter-organelle contact sites with the endoplasmic reticulum (ER). These mitochondrial−ER contacts mediate the release of Ca^{2+} from the ER into mitochondria and cytosol (Wu et al., 2017). As a second messenger, Ca^{2+} unloading promotes mitochondrial homeostasis by regulating organelle dynamics and function (Wu et al., 2017). Mitophagy in cardiomyocytes can also be triggered independent of cytosolic Ca^{2+} levels, oxidative stress, and apoptosis signaling (Quinsay et al., 2010). This action is mediated by the phosphorylation of the BCL-2 interacting protein 3 (BNIP3), which favors its interaction with microtubule-associated proteins 1A/1B light chain 3B (LC3), thereby fueling the mitophagy flux (Liu et al., 2014). Abrogation of c-Jun N-terminal kinase (JNK) signaling and the associated downregulation of BNIP3 have been shown to reverse cardiac remodeling in heart failure (Chaanine et al., 2012).

3. Mitochondrial-derived vesicles: mitophagy add-ins

An ever-growing amount of evidence indicates that, along with mitophagy, an additional process operating via *endo*-lysosomal trafficking contributes to MQC and mitochondrial homeostasis. This route, conserved from bacteria to eukaryotes, signals via vesicles budding. A large set of membranous shuttles is produced and vesicles of mitochondrial origin, named mitochondrial-derived vesicles (MDVs), deliver specific organellar components to late endosome/multivesicular bodies for recycling purposes (Soubannier et al., 2012a). Herein, MDVs are processed, probably according to the nature of their cargo, and released into the extracellular compartment to join the vast array of extracellular vesicles (EVs).

Being a preferential route for the removal of harmful cellular waste, the interest towards the role of EVs and, more specifically, about MDVs in conditions characterized by a decline of cell quality and accrual of intracellular debris has sharply increased (D'Acunzo et al., 2021; Todkar et al., 2021). EVs, beside mediating communication via exchange of

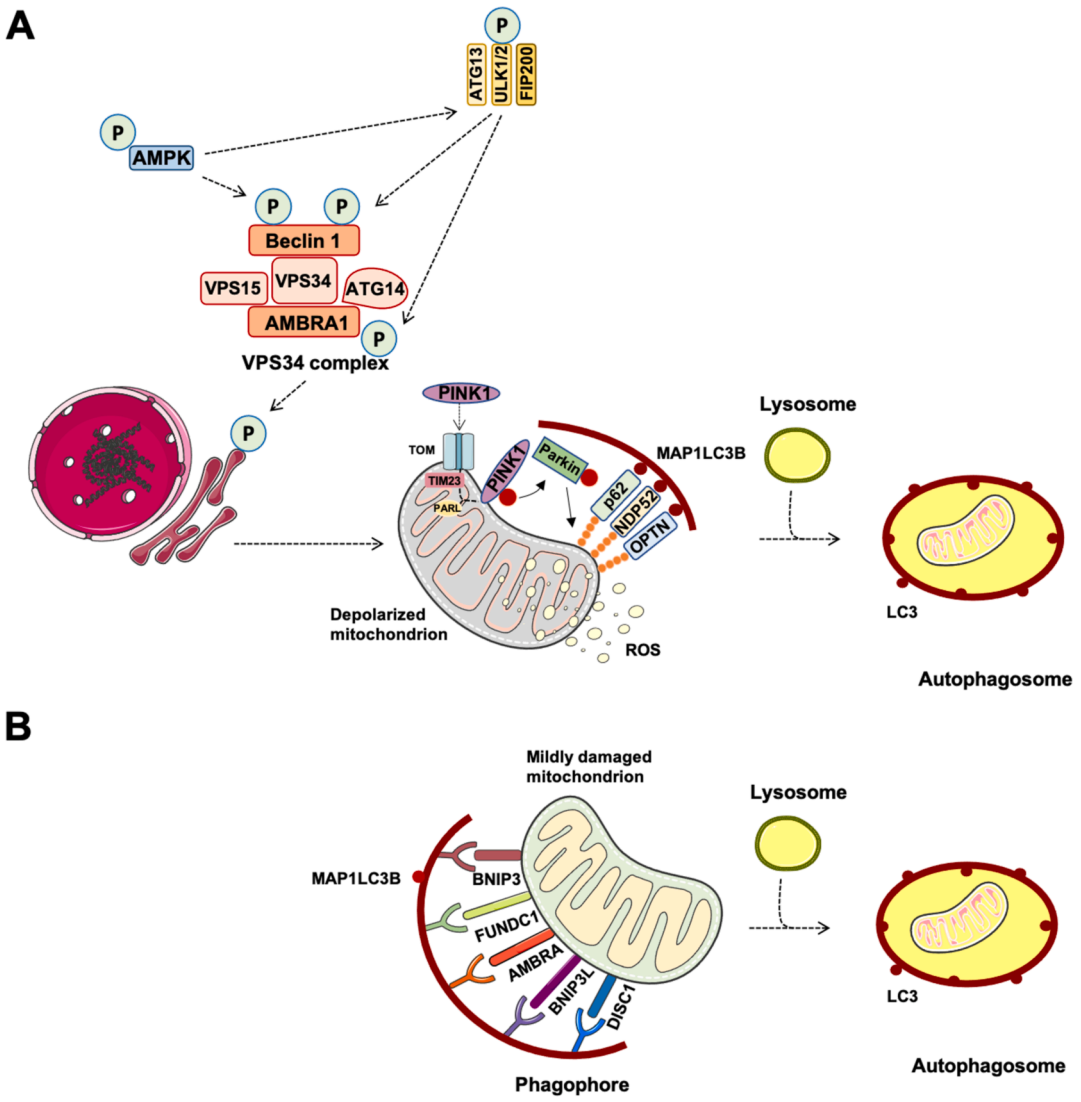

Fig. 1. Schematic representation of quality control mechanisms through mitophagy. A) PINK1/Parkin-dependent mitophagy. The phosphatase and tensin homolog-induced kinase 1 (PINK1) is imported into mitochondria and degraded by presenilin-associated rhomboid-like (PARL) protein through the mediation of the translocase of the outer mitochondrial membrane (TOM) and the translocase of inner mitochondrial membrane 23 (TIM23). In the setting of mitochondrial depolarization, PINK1 is stabilized at the outer mitochondrial membrane (OMM) to trigger the activation of Parkin for the subsequent ubiquitination of mitochondrial components. Finally, the recruitment of a set of autophagy adaptors [i.e., nuclear domain 10 protein 52 (NDP52), optineurin (OPTN), and the sequestosome 1/p62] mediates the engulfment of mitochondria within an autophagosome via the interaction with microtubule-associated protein 1A/1B-light chain 3 (LC3). The endoplasmic reticulum is a source of autophagosome membrane formation via the autophagy core complexes vacuolar protein sorting 34 (VPS34) and unc-51-like kinase 1 (ULK1) that organize autophagosome membrane initiation. Upon completion, autophagosomes fuse with lysosomes to finalize autophagosome's cargo degradation. B) PINK1/Parkin-independent mitophagy. Dysfunctional organelles are coated by the OMM proteins FUN14 domain containing 1 (FUNDC1), autophagy and Beclin-1 regulator 1 (AMBRA1), BCL2-interacting protein 3 like (BNIP3L), BNIP3, and disrupted-in-schizophrenia-1 (DISC1) that help assisting organelles selection and interaction with LC3.

Abbreviations: AMPK, 5' AMP-activated protein kinase; ATG13, Autophagy-related protein 13; ATG14, Autophagy-related protein 14; FIP200, focal adhesion kinase family interacting protein of 200 kDa; VPS15, vacuolar protein sorting 15.

large sets of molecular cargoes (i.e., nucleic acids, proteins, and metabolites) between neighbor cells, are now recognized as transfer systems over a long distance (Edgar, 2016; Shah et al., 2018; Valadi et al., 2007). The ability of EVs to carry organelle components (i.e., MDVs) or even whole mitochondria holding metabolic properties is among the latest extraordinary roles conferred to EVs (D'Acunzo et al., 2021; Todkar et al., 2021).

EVs have been identified in almost all biofluids. The highly heterogeneous set of membranous objects composing the EV population include vesicles that differ in size, function, and biogenesis (Willms et al., 2016). This heterogeneity is reflected by the variety of nomenclature used to refer to EV subtypes. Under the names of shedding vesicles, microvesicles, exosome-like vesicles, nanoparticles, microparticles, and oncosomes is identified the large set of EVs with a diameter of 100-500 nm that are generated by outward budding of the plasma membrane. Exosomes, instead, refer to EVs of endosomal origin with a diameter of 50-150 nm that were initially identified as vesicles released from the plasma membrane during the maturation of reticulocytes (Johnstone et al., 1987). It is now clear that an inward budding of discrete domains of early endosomal membranes generates intraluminal vesicles (ILVs) that evolve into multivesicular bodies (MVBs) (Cocucci and Meldolesi, 2015; Raposo and Stoorvogel, 2013). MVBs are generally degraded into lysosomes for recycling purposes. However, likely depending on cargo information, MVBs can also be re-directed towards

the plasma membrane. Here, MVBs can undergo exocytic fusion and deliver their ILV content (i.e., exosomes) into the extracellular space (Cocucci and Meldolesi, 2015; Raposo and Stoorvogel, 2013) (Fig. 2).

The identification of nucleic acids (mostly RNA) among EV cargo molecules (Valadi et al., 2007) has laid the groundwork for the investigation of EV-associated RNA/DNA as disease markers especially because these molecules may hold potential of tracking down the identity of the originating cell. A role for EV cargoes as biomarkers of defective cellular and MQC systems (Picca et al., 2019a, 2019b, 2020a, 2020b) in conditions characterized by dysfunctional autophagy and accrual of intracellular misfolded proteins is also emerging (Picca et al., 2020e, 2020c). While having a diameter similar to other small EVs (i.e., 70-150 nm), these vesicles are univocally identified by their independence from the core fission GTPase dynamin-related protein 1 and the incorporation of mitochondrial components. MDVs enriched with oxidized cargo have been identified in in-vitro budding assays using bovine heart mitochondria. Although the exact mechanisms of cargo selection and EV incorporation are still unclear, evidence indicates that these vesicles are generated mainly from the outer mitochondrial membrane and, in some cases, may bud out from both outer and inner membranes, thereby incorporating also portions of mitochondrial matrix (McLelland et al., 2014; Neuspiel et al., 2008; Soubannier et al., 2012a, 2012b; Sugiura et al., 2014).

The generation of MDVs occurs under basal physiological conditions and prior to mitochondrial depolarization; a steep increase in their release has been reported in response to mild mitochondrial stressors (Soubannier et al., 2012a, 2012b). To this outcome contributes the coordinated activity of the mitophagy proteins PINK1 and Parkin and mediators of the endocytic pathway (McLelland et al., 2014). Therefore, cells may enact a housekeeping mechanism that complements mitophagy and recycles damaged, but not yet depolarized mitochondria via the release of MDVs (McLelland et al., 2014). A combination of in vitro experiments in cardiac cells and quantitative morphological electron microscopy in vivo has been applied to test the hypothesis that MDV production may be a physiological mechanism for achieving cardiomyocyte homeostasis (Cadete et al., 2016). Results from this investigation indicate that MDV generation disposes mildly oxidized mitochondria independent of mitochondrial depolarization, autophagy signaling, and mitochondrial fission in cardiac cells (Cadete et al., 2016). Indeed, cultured H9c2 myoblasts show constitutive MDV production that acts as a basal housekeeping pathway and occurs even more frequently than mitophagy events (Cadete et al., 2016). The generation of MDVs increases substantially following exposure to mitochondrial stressors (i.e., antimycin-A and xanthine/xanthine oxidase), while both MDV production and mitophagy are enhanced in response to extensive mitochondrial damage (Cadete et al., 2016). Albeit preliminary, these findings indicate that constitutive MDV generation may be crucial for preserving cardiomyocyte homeostasis under physiological conditions and may also serve as a first-line defense against mild stressors. Additional studies clarifying the generalizability of MDV production in various cell types and, most importantly, in vivo are highly sought after. This piece of information is particularly relevant considering the potential of exploiting EVs as tools for the delivery of specific molecules to diseased tissues, thus making these circulating cellular boxes potential targets for therapeutic development (Picca et al., 2020d).

4. Cell-free mtDNA: mitochondrial signaling beyond organelle's boundaries

Nucleic acids, including genomic DNA, mitochondrial DNA (mtDNA), viral DNA, and RNA (e.g., mRNA and microRNAs) may be retrieved in the circulation as cell-free molecules (Helmig et al., 2015). High circulating levels of nucleic acids have been identified in several conditions, including CVD (González-Masiá et al., 2013; Suzuki et al., 2008). The molecular mechanisms mediating their cellular release are unclear (Muotri et al., 2007). However, their unloading mostly occurs from injured tissues/cells or apoptotic and immune activated cells (Jahr et al., 2001), while a smaller portion of derives from neutrophil extracellular trap release, phagocytosis, and oncosis (Thierry et al., 2016). Of all cell-free DNA subpopulations, mtDNA molecules are those mostly represented and have been indicated as prognostic biomarkers in a vast array of conditions, including CVD (Helmig et al., 2015; Liu et al., 2015). The evaluation of circulating levels of mtDNA has theoretical advantages in the setting of CVD over other cell-free DNA species that are likely involved in different pathological processes (e.g., tumor biomarker).

MtDNA is packaged within the mitochondrial matrix and encodes for components of electron transport chain (ETC) complexes that are in charge of regulating cellular bioenergetics via oxidative phosphorylation (OXPHOS) (Reinecke et al., 2009). The mitochondrial genome is highly polymorphic in humans and mitochondrial haplogroups are geographically-specific mitochondrial genetic variants harboring specific polymorphisms (Torroni et al., 1992). These polymorphisms generate small amino acid changes into ETC complexes that translate in varying OXPHOS efficiency. In some cases, these differences lead to mitochondrial dysfunction and impaired ATP production that, together with low membrane potential and ROS accrual, result in oxidative damage (Judge and Leeuwenburgh, 2007).

Similar to matrix mitochondrial enzyme activities (e.g., citrate synthase), mtDNA levels are proxy for the bioenergetic potential and the

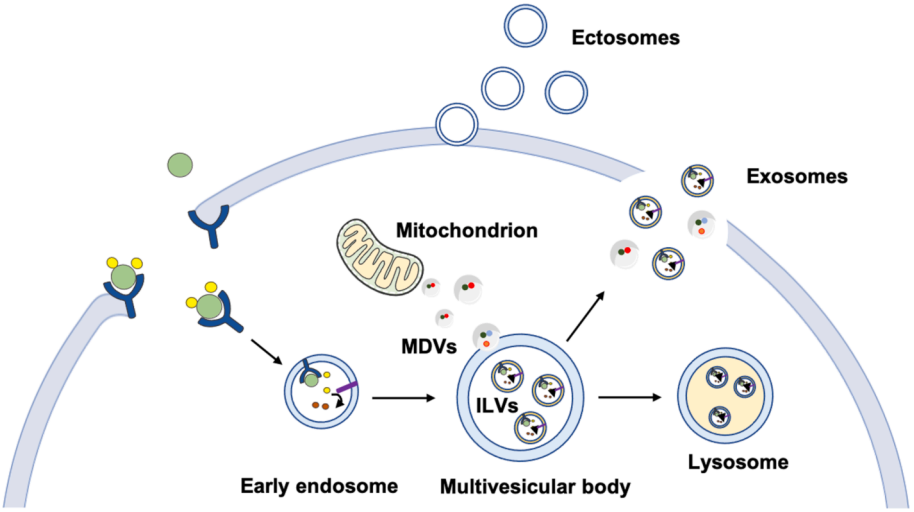

Fig. 2. Mitochondria quality control through the generation and release of mitochondrial-derived vesicles. The generation of mitochondrial-derived vesicles (MDVs) has been described as a form of piecemeal mitophagy that operates as an alternative to the canonical degradative pathway to dispose mildly oxidized mitochondria. These organelles are targeted by the canonical phosphatase and tensin homolog-induced kinase 1/Parkin degradative pathway which, in conjunction with the formation of localized membrane curvatures driven by oxidized cardiolipin and other unknown proteins, generates MDVs. These vesicles reach out and are processed along the endo-lysosomal system whereby they form multivesicular bodies and are subsequently extruded as exosomes. *Abbreviation*: ILVs, intraluminal vesicles.

mitochondrial mass of a cell/tissue (Lightowlers et al., 1997; Malik and Czajka, 2013). Under physiological conditions, mtDNA replicates within the organelle and its integrity is ensured via MQC. However, in the setting of defective mitophagy, mtDNA degradation and recycling of damaged molecules become inefficient and mtDNA follows unconventional routes to be disposed (Picca et al., 2021). Emerging evidence indicates that cell-free mtDNA may not only trigger inflammation, coagulation and immunity, but also induce cell death and tissue damage. In particular, cell-free mtDNA has been directly involved in early endothelial dysfunction and vasculopathies, both relevant to the development of CVD (Bhagirath et al., 2015). On the other hand, cell-free mtDNA may contribute to endogenous repair systems via regulation of stem cell activation, fate decisions, and defense against cellular senescence (Zhang et al., 2018).

Cardiac metabolism is almost entirely aerobic and mtDNA variants may affect heart function and predispose cardiomyocytes to mitochondrial dysfunction (Judge and Leeuwenburgh, 2007). Specific mitochondrial haplogroups have been associated with aging (Domínguez-Garrido et al., 2009), VO_2 max (Martínez-Redondo et al., 2010), and the development of diseases including cardiomyopathies (Fernández-Caggiano et al., 2013, 2012; Govindaraj et al., 2014; Hagen et al., 2013). The link between mtDNA alterations and CVD may reside in the proportion of ROS being produced. Indeed, oxidative damage to mtDNA along with oxidation of proteins involved in intracellular Ca^{2+} homeostasis have been indicated as major triggers of atrial fibrillation (Ai et al., 2005; Lin et al., 2003). As a consequence of protein oxidation, the release of higher levels of Ca^{2+} from the ER (Ca^{2+} sparks) into the cytoplasm or from mitochondria has been identified in cardiac myocytes. Under these circumstances, abnormal electrophysiological changes, including altered cardiac excitation-contraction coupling, arrhythmogenesis, and automatism occur (Bers, 2005; Hove-Madsen et al., 2004). High levels of oxidized mtDNA have been found in atrial myocardium of patients with atrial fibrillation with concomitant increase of mtDNA content (Lin et al., 2003). Moreover, a number of mtDNA mutations have been identified in patients with atrial fibrillation, such as the large 4977-bp and short 9-bp deletions as well as heteroplasmic mutations (Lin et al., 2003; Park et al., 2007). These findings indicate that the increase in mtDNA oxidative damage and deletions observed in the myocardium of people with atrial fibrillation may contribute to impairment of mitochondrial bioenergetics and trigger an oxidative vicious circle that ultimately causes atrial myopathy and leads to atrial fibrillation. The relationship between mtDNA content and the occurrence of atrial fibrillation has been investigated in patients enrolled in the Atherosclerosis Risk in Communities project. Results from this study indicate the existence of an inverse association between mtDNA copy number and the risk of atrial fibrillation, independent of traditional cardiovascular risk factors (Zhao et al., 2020). Recent findings have also shown a relationship between mitochondrial haplogroups and the risk of atrial fibrillation (Roselló-Díez et al., 2021).

Finally, focal myocardial necrosis due to myocardial infarction has been associated with high levels of mtDNA (Bliksoen et al., 2012). This increase in cell free mtDNA was also indicated to contribute to local and systemic inflammation following myocardial infarction (Bliksoen et al., 2012).

5. Is mitophagy a therapeutic target in cardiac aging? State of the art and future perspectives

The accrual of dysfunctional mitochondria is a well-established phenotypic alteration of the aged heart and evidence indicates that mitophagy impairment is a major contributor to organelle dyshomeostasis and tissue dysfunction (Eisenberg et al., 2016; Inuzuka et al., 2009; Ren et al., 2017; Wang et al., 2019). Genetic and pharmacological interventions targeting mitophagy have shown great potential towards extending lifespan in preclinical models (Table 1) (Ryu et al., 2016; Schiavi et al., 2015).

In particular, the overexpression of Parkin in *Drosophila* has been reported to enhance the turnover of defective mitochondria via shifting mitochondrial dynamics towards fission and to slow aging (Rana et al., 2013). Parkin overexpression in the heart of old mice has also been shown to promote the incorporation of defective mitochondrial into autophagosomes and alleviate age-related cardiac functional decline (Hoshino et al., 2013). The oral administration of the mitophagy inducer urolithin A, a natural compound derived as a gut metabolite of ellagic acid, has been reported to extend lifespan in *C. elegans* and improve muscle function in rodents (Ryu et al., 2016). Moreover, dietary administration of the natural polyamine spermidine to old mice has been shown to reduce cardiac hypertrophy and preserve diastolic function via the promotion of cardiomyocyte mitophagy and mitochondrial respiration (Eisenberg et al., 2016). Of note, this cardioprotective effect was absent in cardiomyocytes lacking the autophagy-related protein ATG5 (Eisenberg et al., 2016). Some evidence indicates that high levels of

Table 1
Summary of studies that used genetic and pharmacological interventions targeting quality control pathways to counteract cardiac aging.

Species	Intervention(s)	Targeted pathway(s)	Functional implications	Reference
C. elegans	Urolitin A	Mitophagy	Attenuation of cardiac aging	Ryu et al., 2016
D. melanogaster	Parkin overexpression	Mitochondrial dynamics	Attenuation of cardiac aging	Rana et al., 2013
Mouse	Short-term calorie restriction/ rapamycin administration	Protein oxidation and ubiquitination	Reversion of age-dependent cardiac hypertrophy and diastolic dysfunction	Dai et al., 2014
Mouse	Spermidine	Mitophagy and mitochondrial respiration	Reduction of cardiac hypertrophy and preservation of diastolic function	Eisenberg et al., 2016
Mouse	Parkin overexpression	General autophagy	Alleviation of age-related cardiac functional decline	Hoshino et al., 2013
Mouse	Tuberin (TSC2) knock-in mutations	Mammalian target of rapamycin (mTORC1)	Cardiac protection against pressure-overload	Ranek et al., 2019
Mouse	Pharmacological and genetic inhibition of mTORC1	Ras homology enriched in brain (RHEB) and mTORC1	Reduction of myocardial damage during ischemia, especially in obese patients	Sciarretta et al., 2012
Mouse	TAT-Beclin 1	General autophagy /mitophagy	Attenuation of heart mitochondrial dysfunction during pressure overload	Shirakabe et al., 2016
Mouse	Calorie restriction	Cell scaffolding and apoptosis	Prevention of aging cardiomyopathy	Yan et al., 2013
Mouse	Swimming exercise	Mitochondrial quality control and apoptosis	Improvements of post-myocardial infarction cardiac remodeling	Zhao et al., 2018
Mouse	Dasatinib, quercetin, and navitoclax	Senescent cells	Restoration of vascular endothelial function	Zhu et al., 2015
Rabbit	Inhibitors of histone deacetylase	General autophagy	Reduction of myocardial infarct size during ischemia-reperfusion	Xie et al., 2014
Rat	Enalapril	Mitochondrial quality control	Mitigation of age-dependent cardiac hypertrophy and oxidative damage	Picca et al., 2018b
Rat	Exercise preconditioning	Mitophagy	Cardioprotection through mitohormesis	Yuan et al., 2018
Rat	Exercise preconditioning	General autophagy	Alleviation of exercise-induced myocardial injury	Wan et al., 2021

dietary spermidine correlate with reduced blood pressure a lower incidence of CVD also in humans (Eisenberg et al., 2016).

The preservation of efficient MQC to maintain a healthy mitochondrial network is also an attractive therapeutic approach in the treatment of CVD (Bertero and Maack, 2018; Murphy et al., 2016). In particular, promotion of autophagy has been linked to mitochondrial function and cardiovascular homeostasis (Bertero and Maack, 2018; Murphy et al., 2016). Lifestyle (i.e., nutrition and physical activity) and pharmacological interventions targeting autophagy are emerging candidates that could be harnessed for the stimulation of general autophagy in the cardiovascular system. Short-term calorie restriction or the administration of rapamycin, a known inhibitor of the mammalian target of rapamycin (mTOR), has also shown to trigger general autophagy and reverse myocardial ischemia, cardiac hypertrophy, and cardiac aging (Dai et al., 2014; Ranek et al., 2019; Sciarretta et al., 2012; Yan et al., 2013). Furthermore, the cell-permeable ATG6/Beclin1-derived autophagy-inducing peptide (TAT-Beclin-1) has been shown to promote autophagy/mitophagy by mobilizing the endogenous protein Beclin-1 and to attenuate mitochondrial dysfunction in the heart during pressure overload (Shirakabe et al., 2016). The administration of the angiotensin converting enzyme inhibitor enalapril to old rats has been indicated to offer cardioprotection during aging by mitigating age-dependent cardiac hypertrophy and oxidative damage (Picca et al., 2018b). This effect was associated with increased mitochondrial mass, mitochondriogenesis and MQC signaling (Picca et al., 2018b). Finally, inhibitors of histone deacetylase have been tested for the ability of blunting I-R injury and have shown cardioprotective effect including reduction of myocardial infarct size during I-R at least in part by inducing autophagy (Xie et al., 2014).

The activation of mitophagy by exercise has a key role for its adaptative responses (Guan et al., 2019; Marquez and Han, 2017). Acute and chronic exercise modulates the activity of LC3 I and its autophagosome-membrane-associated lipidated form LC3 II and disrupt the p62 complex in different tissues (Chen et al., 2018; He et al., 2012; Yuan et al., 2018; Zhao et al., 2018). In particular, early exercise preconditioning has a protective role on acute cardiovascular stress via translocation of BNIP3 to mitochondria and the recruitment of the mitophagy effector LC3 (Yuan et al., 2018). These findings have recently been confirmed and expanded by Wan et al. (2021), who reported that exercise preconditioning prevented cardiac injury by modulating the transcription and translation of many LC3 lipidation-associated proteins. In one of the few studies investigating the chronic effects of exercise, Zhao et al. (2018) observed that swimming-training-induced improvements in post-myocardial infarction cardiac remodeling was accompanied by reduced LC3-II and p62 levels and increased PINK/Parkin expression. In contrast, BCL2 knockout mice, that are deficient in stimulus-induced but not basal autophagy, show marked impairment in exercise-induced mitophagy, which is accompanied by reduced maximal exercise capacity and glucose tolerance (He et al., 2012).

Taken as a whole, these findings indicate that the regulation of autophagy/mitophagy may be a therapeutic strategy for treating or alleviating the burden of CVD. However, a major challenge is the definition of more specific targets of the autophagy/mitophagy pathway in relation to CVD, considering that autophagy stimulators may also regulate other critical cellular processes. Furthermore, under certain conditions, excessive mitophagy may be detrimental (Liu et al., 2013; Saito and Sadoshima, 2015). Indeed, the critical window within which the stimulation of mitophagy becomes cardioprotective instead of inducing further damage especially in the context of compromised quality control processes is yet to be defined. Furthermore, it will be crucial to clarify whether triggering mitophagy may be a valuable therapeutical approach once cardiac mitochondrial damage has accumlated. The development of techniques that provide robust measurement of mitophagy in vivo as well as methods that allow evaluating the autophagy/mitophagy flux in humans are necessary to answer these research questions (Klionsky et al., 2021).

Finally, genetic models have revealed that the accumulation of senescent cells contribute to the pathophysiology of cardiovascular aging and promotes CVD progression via the expression of a proinflammatory and profibrotic senescence-associated secretory phenotype (SASP) factors (Dookun et al., 2020). Based on these findings, therapeutics able to induce the selective elimination of senescent cells via apoptosis have been developed. These senescent cell-targeted apoptotic inducer compounds are termed senolytics and their potential to ameliorate age-associated CVD is currently under investigation (Dookun et al., 2020). In particular, dasatinib, quercetin, and navitoclax have shown great potential towards the attenuation or prevention of CVD mostly via restoration of vascular endothelial function (Zhu et al., 2015). In particular, the interdependent regulation of senescence and mitophagy warrants investigation to develop novel therapeutics that may overcome the side effects of these drugs.

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