Received: 24 December 2019 | Revised: 31 March 2020 | Accepted: 1 April 2020

DOI: 10.1111/bph.15068

Themed Issue: Cellular metabolism and diseases

REVIEW ARTICLE

The role of mitochondrial dynamics in cardiovascular diseases

Maurizio Forte[1] | Leonardo Schirone[2,3] | Pietro Ameri[4,5] | Cristina Basso[6] | Daniele Catalucci[7,8] | Jessica Modica[7,8] | Cristina Chimenti[9] | Lia Crotti[10,11,12] | Giacomo Frati[1,2] | Speranza Rubattu[1,13] | Gabriele Giacomo Schiattarella[14,15] | Daniele Torella[16] | Cinzia Perrino[15] | Ciro Indolfi[16] | Sebastiano Sciarretta[1,2] | Italian Society of Cardiology Working group on Cellular and Molecular Biology of the Heart

[1]Department of AngioCardioNeurology, IRCCS Neuromed, Pozzili, Italy

[2]Department of Medical and Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy

[3]Department of Internal, Anesthetic and Cardiovascular Clinical Sciences, "La Sapienza" University of Rome, Rome, Italy

[4]Cardiovascular Disease Unit, IRCCS Ospedale Policlinico, Genova, Italy

[5]Department of Internal Medicine, University of Genova, Genova, Italy

[6]Cardiovascular Pathology Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua Medical School, Padova, Italy

[7]Humanitas Clinical and Research Center, IRCCS, Rozzano, Italy

[8]National Research Council, Institute of Genetic and Biomedical Research - UOS, Milan, Italy

[9]Department of Cardiovascular, Respiratory, Nephrologic, and Geriatric Sciences, Sapienza University of Rome, Rome, Italy

[10]Istituto Auxologico Italiano, IRCCS, Center for Cardiac Arrhythmias of Genetic Origin and Laboratory of Cardiovascular Genetics, Milan, Italy

[11]Istituto Auxologico Italiano, IRCCS, Department of Cardiovascular, Neural and Metabolic Sciences, San Luca Hospital, Milan, Italy

[12]Department of Medicine and Surgery, Università Milano-Bicocca, Milan, Italy

[13]Department of Clinical and Molecular Medicine, School of Medicine and Psychology, Sapienza University of Rome, Rome, Italy

[14]Department of Internal Medicine (Cardiology), University of Texas Southwestern Medical Center, Dallas, TX, USA

[15]Division of Cardiology, Department of Advanced Biomedical Sciences, Federico II University of Naples, Naples, Italy

[16]Molecular and Cellular Cardiology Laboratory, Department of Experimental and Clinical Medicine, Magna Graecia University, Catanzaro, Italy

Correspondence
Sebastiano Sciarretta, Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy.
Email: sebastiano.sciarretta@uniroma1.it

The process of mitochondrial dynamics is emerging as a core player in cardiovascular homeostasis. This process refers to the co-ordinated cycles of biogenesis, fusion, fission and degradation to which mitochondria constantly undergo to maintain their integrity, distribution and size. These mechanisms represent an early response to mitochondrial stress, confining organelle portions that are irreversibly damaged and preserving mitochondrial function. Accumulating evidence demonstrates that impairment in mitochondrial dynamics leads to myocardial damage and cardiac disease progression in a variety of disease models, including pressure overload, ischaemia/reperfusion and metabolic disturbance. These findings suggest that modulation of mitochondrial dynamics may be considered as a valid therapeutic strategy in

Abbreviations: Afg3l1, mAAA protease complex ATPase family gene-3 yeast-like-1; CVDs, cardiovascular diseases; Drp1, dynamin-related protein 1; ETC, electron transport chain; Fis1, mitochondrial fission protein 1; Fundc1, FUN14 domain-containing protein 1; GDP, guanosine diphosphate; GTP, guanosine-5′-triphosphate; IMM, inner mitochondrial membrane; I/R, ischaemia/reperfusion; Mdv1, mitochondrial division protein 1; Mfn 1-2, mitofusin 1 and 2; Mff, mitochondrial fission factor; mtPTP, mitochondrial permeability transition pore; Oma1, mitochondrial metalloendopeptidase; OMM, outer mitochondrial membrane; Opa1, optic atrophy protein 1; Parkin, E3 ubiquitin ligase; PINK1, phosphatase and tensin homologue (PTEN)-induced kinase 1; PGC1, PPAR γ coactivator 1; SNX9, Sorting nexin 9; Yme1l1, ATP-dependent zinc metalloprotease..

Maurizio Forte and Leonardo Schirone equally contributed to the work.

cardiovascular diseases. In this review, we discuss the current evidence about the role of mitochondrial dynamics in cardiac pathophysiology, with a particular focus on the mechanisms underlying the development of cardiac hypertrophy and heart failure, metabolic and genetic cardiomyopathies, ischaemia/reperfusion injury, atherosclerosis and ischaemic stroke.

LINKED ARTICLES: This article is part of a themed issue on Cellular metabolism and diseases. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v178.10/issuetoc

1 | INTRODUCTION

Mitochondria are highly dynamic organelles devoted to the production of adenosine triphosphate (ATP), as a result of oxidative phosphorylation (Forte, Palmerio, Bianchi, Volpe, & Rubattu, 2019). Mitochondria also represent the main intracellular source of ROS, produced during oxidative phosphorylation processes (Forte, Palmerio, et al., 2019; Saito & Sadoshima, 2015). In the last two decades, mitochondrial dysfunction has emerged as one of the main pathogenic mechanisms underlying the development of an increasing number of diseases, including cardiovascular diseases (CVDs). Dysfunctional mitochondria limit energy production, increase ROS generation and send apoptotic signals. These events exacerbate mitochondrial damage, by creating a vicious circle resulting in tissue damage and dysfunction (Forte, Palmerio, et al., 2019).

Mitochondrial homeostasis is regulated by specific mechanisms that preserve organelle structure and function (Vasquez-Trincado et al., 2016). Among these mechanisms, mitochondrial dynamics plays a major role and include three major events: mitochondrial biogenesis, fission and fusion. These processes are also tightly coupled with mitochondrial degradation by autophagy, also named mitophagy. The underlying molecular machinery is strongly interconnected, ensuring the preservation of mitochondrial morphology, integrity and content (Saito & Sadoshima, 2015). Mitochondrial fusion allows a dynamic repair of reversibly damaged portions of mitochondria, forming functional elongated organelles (Saito & Sadoshima, 2015; Sciarretta, Maejima, Zablocki, & Sadoshima, 2018). Conversely, mitochondrial fission occurs when mitochondria are irreversibly damaged and potentially harmful for the cell. Mitochondrial fission is characterized by the fragmentation of the damaged mitochondrion into small sphere-shaped organelles that are then isolated and digested by mitophagy (Saito & Sadoshima, 2015; Sciarretta et al., 2018). Moreover, mitochondrial fission ensures the correct division of mitochondria in the daughter cells during mitosis. In physiological conditions, mitochondria continuously undergo cycles of fusion and fission, and various proteins have been found to regulate these processes and will be discussed in this review. When fission and fusion are balanced, mitochondrial size is homogeneous.

Mitochondrial dynamics often represents the first response against mitochondrial stress, such as changes in mitochondrial membrane potential ($\Delta\Psi m$). Mitochondrial dynamics is particularly important in cells with higher energy demand, for example, cardiomyocytes, which continuously require ATP to support cardiac function (Saito & Sadoshima, 2015; Vasquez-Trincado et al., 2016). In fact, increasing lines of evidence suggest that the unbalance of mitochondrial dynamics may contribute to the onset or progression of several cardiac diseases. For example, mice lacking mitochondrial fission develop severe cardiac abnormalities (Ikeda et al., 2015; Shirakabe et al., 2016; Song et al., 2015). In the heart, loss of functions models of single genes regulating fission or fusion revealed the importance of these mechanisms in maintaining cardiac homeostasis at baseline and during stress, for example, pressure and volume overload, cardiac ischaemia/reperfusion injury, genetic and metabolic cardiomyopathy (Sciarretta et al., 2018). Interestingly, impairing both fusion and fission together kept mitochondrial dynamics balanced and showed modest phenotypic effects (Song, Franco, Fleischer, Zhang, & Dorn, 2017).

In this review, we discuss the molecular biology underlying mitochondrial dynamics; highlight how defective mitochondrial dynamics may contribute to cardiovascular damage during stress; elaborate upon the importance of considering mitochondrial dynamics as an emerging therapeutic target in CVDs.

2 | BIOLOGY OF MITOCHONDRIAL DYNAMICS

2.1 | Mitochondrial fusion

The process of mitochondrial fusion comprises the union of both outer (OMM) and inner mitochondrial membranes (IMM) of two merging organelles. The main proteins orchestrating this process in mammalian cells are nuclear-encoded and belong to the family of dynamin-related GTPases (Figure 1). These include mitofusin 1 (Mfn1), mitofusin 2 (Mfn2) and optic atrophy 1 protein (Opa1) (Cipolat, Martins de Brito, Dal Zilio, & Scorrano, 2004). Mfn1 and Mfn2 mediate outer membrane fusion, whereas Opa1 is required for the fusion of the inner mitochondrial membranes. Mfn1/2 exert redundant functions and a decrease in the expression of one isoform may be compensated by the overexpression of the other one (Chan, 2006). Mfn1/2 pair with their homologues located at the outer mitochondrial membranes of the other involved mitochondrion, then GTP is hydrolysed generating a power stroke that is critical for

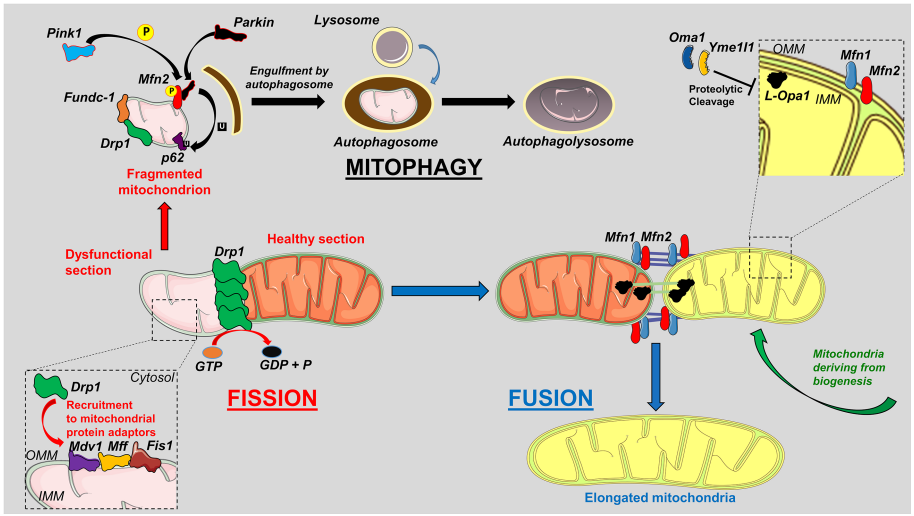

FIGURE 1 Schematic representation of mitochondrial dynamics. Mitochondrial fission leads to fragmented and spherical mitochondria that can be removed by mitophagy (left side). In mitochondrial fusion (right panel), reversible damaged mitochondria fuse with new freshly formed mitochondria by biogenesis. In the figure, the main proteins orchestrating mitochondrial dynamics are highlighted. See the text for further details. Legend: Ca^{++}, calcium; Drp1, dynamin-related protein 1; Fis1, mitochondrial fission protein 1; Fundc1, FUN14 domain-containing protein 1; GDP, guanosine diphosphate; GTP, guanosine-5′-triphosphate; IMM, inner mitochondrial membrane; Mdv1, mitochondrial division protein 1; Mfn 1-2, mitofusin 1 and 2; Mff, mitochondrial fission factor; mtPTP, mitochondrial permeability transition pore; Oma1, mitochondrial metalloendopeptidase; OMM, outer mitochondrial membrane; Opa1, optic atrophy protein 1; P, phosphate group; parkin, E3 ubiquitin ligase; Yme1l1, ATP-dependent zinc metalloprotease. The figure was made in part using tools provided by Servier Medical Arts

membrane fusion (Filadi, Pendin, & Pizzo, 2018). Conversely, Opa1 GTPase is located at the inner mitochondrial membranes, regulates mitochondrial fusion and also participates in cristae remodelling in response to energetic stress or mitochondrial damage, thereby preserving inner mitochondrial membranes integrity and function. Opa1 undergoes proteolytic cleavage changing from a long-anchored isoform (L-Opa1) to a short one (S-Opa1). Oligomerization of a S-Opa1 isoform with two L-Opa1 subunits forms a complex that tightens cristae and boosts oxidative phosphorylation by increasing ATP synthase assembly. Interestingly, these processes seemed to be independent, considering that an Opa1 mutant that can oligomerize but has no fusion activity is still able to maintain cristae structure (Patten et al., 2014). The proteolytic cleavage of Opa1 is mediated by different proteins: mitochondrial metalloendopeptidase Oma1, ATP-dependent zinc metalloprotease Yme1L1, presenilin-associated rhomboid-like (Parl) protein, paraplegin and mAAA protease complex ATPase family gene-3 yeast-like-1 (Afg3l1) (Anand et al., 2014). The acetylation of lysine residues is an important post-translational modification controlling Opa1 activity. Cardiac stress triggers Opa1 hyperacetylation, which is associated with reduced GTPase activity. This effect is counteracted by sirtuin 3 (Sirt3) deacetylase, which directly binds Opa1 and promotes mitochondrial function and dynamic networking, in addition to its already known positive effects on mitochondrial metabolic enzymes (Samant et al., 2014). Generally, L-Opa1 is required for mitochondrial fusion and it is also involved in maintaining mitochondrial respiration. In contrast, increased activity of Oma1 leads to S-Opa1 accumulation in the intermembrane space, which contributes to shift mitochondrial dynamics towards fission (MacVicar & Langer, 2016; Varanita et al., 2015). Systemic deletions of Mfn1/2 and Opa1 are embryonically lethal in mice (Chen et al., 2003; Davies et al., 2007), demonstrating their fundamental importance during embryogenesis and early development. In humans, mutations in Opa1 are the cause of optic atrophy, Behr syndrome and genetic forms of encephalomyopathy (Bonneau et al., 2014; Spiegel et al., 2016). Likewise, mutations in the Mfn2 gene have been associated with Charcot–Marie–Tooth disease type 2A (CMT2A), a severe neurodegenerative disorder (Zuchner et al., 2004).

2.2 | Mitochondrial fission

Mitochondrial fission is mostly regulated by dynamin-related protein 1 (Drp1) (Figure 1). Once activated, Drp1 translocates from the cytosol to mitochondria, despite no mitochondrial targeting motifs have been identified in Drp1 primary sequence (Bossy-Wetzel, Barsoum, Godzik, Schwarzenbacher, & Lipton, 2003). Indeed, Drp1 recruitment to the mitochondria is facilitated by specific adaptor proteins located in the outer mitochondrial membranes, such as mitochondrial fission 1 protein (Fis1), mitochondrial division protein 1 (Mdv1) and mitochondrial fission factor (Mff) (Saito & Sadoshima, 2015). Once recruited to the outer mitochondrial membranes, Drp1 undergoes GTP-dependent oligomerization forming a spiral ring of 20 nm of inner diameter (Basu et al., 2017). This process consists in tubing each of the four 7 nm thick juxtaposed mitochondrial membranes into a narrow lumen, which forces a reorganization that was shown to involve proteins such as dynamin 2, endophilin 1 and sorting nexin 9 (SNX9) (Loson, Song, Chen, & Chan, 2013). Drp1 recruitment and oligomerization are regulated by post-translational modifications, such

as ubiquitination, phosphorylation, sumoylation and S-nitrosylation (van der Bliek, Shen, & Kawajiri, 2013). For example, **protein kinase A** reduces Drp1 activity whereas kinase **Cdk1**/cyclin B increases it both during mitosis and early stress response (Chang & Blackstone, 2007a; Taguchi, Ishihara, Jofuku, Oka, & Mihara, 2007). The serine/threonine kinase **Pim1** was reported to inhibit Drp1 activity by phosphorylation at serine 637 (S637) (Chang & Blackstone, 2007b; Din et al., 2013; Kim et al., 2011). Conversely, calcineurin dephosphorylates Drp1, thereby increasing its activity (Wang et al., 2011). Ubiquitination by the E3-ubiquitin ligase, parkin was also found to negatively regulate Drp1, promoting its proteasomal degradation (Lutz et al., 2009; Wang et al., 2011). Drp1 also undergoes sumoylation, a post-translation modification which generally increases its activity. The mitochondrial-anchored protein ligase (MAPL), a small ubiquitin-like modifier ligase (SUMO)-3, was reported to enhance mitochondrial fission (Prudent et al., 2015). Conversely, studies on different members of the sentrin-specific protease (SENP) family, which reverse SUMO conjugation in mammalian cells, provided divergent results. In fact, it was shown that SENP-3 desumoylated Drp1 and reduced Drp1-induced cell death in ischaemic conditions (Guo et al., 2013). In contrast, cardiac overexpression of SENP5 induced apoptosis and cardiomyopathy in mice (Kim et al., 2015). Further investigations are required to define the role of Drp1 sumoylation in heart pathophysiology.

Drp1 is involved in the regulation of mitophagy and mitochondrial permeabilization, which ultimately triggers the intrinsic apoptotic pathway (Zhang et al., 2017). It was also demonstrated that Drp1 co-localizes with the proapoptotic factor **Bax** and its down-regulation inhibits both fission and apoptosis (Karbowski et al., 2002). However, other studies reported that Bax-induced apoptosis is not associated with fission activation and that **Bcl-xL** overexpression blocks apoptosis without inhibiting mitochondrial fission (Sheridan, Delivani, Cullen, & Martin, 2008). In humans, point mutations of Drp1 are sufficient to cause fatal abnormal brain development. Similarly, deletions of Drp1 are lethal in *Caenorhabditis elegans* (Labrousse, Zappaterra, Rube, & van der Bliek, 1999; Waterham et al., 2007).

2.3 | Interaction between mitochondrial dynamics and mitophagy

Mitophagy is the selective form of autophagy devoted to the digestion of senescent or irreversibly damaged mitochondria (Saito & Sadoshima, 2015). A co-ordinated fission-increase response is critical to remove damaged mitochondrial portions, forming spherical and unhealthy mitochondria that are cleared by mitophagy (Sciarretta et al., 2018). Mitophagy may occur through parkin-dependent or parkin-independent mechanisms. In parkin-mediated mitophagy, the phosphatase and tensin homologue (PTEN)-induced kinase 1 (**PINK1**) recruits parkin at the outer mitochondrial membranes. This may involve the direct phosphorylation of parkin or other indirect mechanisms, for example, PINK1 phosphorylation of Mfn2, which is recognized by parkin, which subsequently localizes to the mitochondria (Chen & Dorn, 2013). The involvement of Mfn2 as a parkin receptor is a further element that suggests the interplay between mitochondrial dynamics and mitophagy. Once activated, parkin ubiquitinates different targets allowing their interaction with mitophagy adaptors, for example, p62/sequestosome 1 (Durcan et al., 2014). Then, the interaction of the recruited sequestosome 1 with microtubule-associated protein 1 light chain 3 (LC3) mediates the engulfment of damaged mitochondria into autophagosomes (Sciarretta et al., 2018). Finally, the sequestered mitochondria are digested by the lysosomes fusing with autophagosomes, forming the so-called 'autolysosomes'. Parkin activity is also counteracted by several mechanisms, including inhibition of its translocation to the outer mitochondrial membranes by p53 and deubiquitination of its targets by USP15 and USP30 on outer mitochondrial membranes proteins (Hoshino et al., 2013; Sciarretta et al., 2018).

Mitophagy may also occur through parkin-independent mechanisms, for example, those mediated by Bcl2-like protein 13 (Bcl2-L-13), Bcl2/adenovirus E1B 19-kDa protein-interacting protein 3 (**Bnip3**), NIX, cardiolipin or FUN14 domain-containing protein 1 (Fundc-1), which are the better characterized so far (Saito & Sadoshima, 2015; Sciarretta et al., 2018). Besides mitophagy activation, Fundc-1 is also able to regulate mitochondrial fusion and fission by interacting with Opa1 and Drp1, respectively, located at the outer mitochondrial membranes. Fundc-1 interaction with Opa1 inhibits both mitophagy and fission. In contrast, Fundc-1 association with Drp1 promotes these processes (Chen et al., 2016).

Accumulating lines of evidence demonstrate that fission inhibition usually leads to impaired mitophagy (Twig et al., 2008). In particular, it was reported that mitochondrial fission generates an uneven population of mitochondria characterized by reduced membrane potential and decreased levels of the fusion protein Opa1. Reduced fusion induced by Opa1 decrease also contributes to segregate dysfunctional mitochondria so that they can easily be detected and removed by mitophagy (Twig et al., 2008). Contemporary cardiac deletion of Mfn1 and Mfn2 induced the accumulation of defective mitochondria in the heart, without a concomitant increase of mitophagy (Chen, Liu, & Dorn, 2011; Song, Mihara, Chen, Scorrano, & Dorn, 2015). This suggests a fundamental role of mitochondrial fusion in the regulation of mitochondrial quality control. However, single genetic deletion of Mfn1 had a little effect on cardiomyocytes mitochondria whereas single Mfn2 deletion caused the accumulation of enlarged mitochondria (Chen & Dorn, 2013) and impaired autophagosome-lysosome fusion, which is the last step of the autophagic flux (Zhao et al., 2012). These results suggest that Mfn2, but not Mfn1, plays a crucial role in mitochondrial quality control, including mitophagy. As mentioned above, Mfn2 can be phosphorylated by Pink1, an event that facilitates Parkin recruitment to the outer mitochondrial membranes, thereby promoting parkin-dependent mitophagy. Interestingly, decreased mitochondrial fusion by mitofusin inhibition limited cardiomyopathy in a *Drosophila* model of parkin gene deletion, which displayed mitophagy impairment (Bhandari, Song, Chen, Burelle, & Dorn, 2014). In fact, parkin deficiency contributed to the fusion between dysfunctional mitochondria (not removed by mitophagy) and healthy ones. Similarly, Drp1-mediated fission was also shown to counteract non-specific

parkin phosphorylation, protecting undamaged mitochondria from unnecessary and mistargeted Pink1-Parkin pro-autophagic activity (Burman et al., 2017). Interestingly, Bnip3 overexpression in cardiomyocytes induced Drp1 activation and increased autophagy, whereas Drp1 inhibition was sufficient to counteract the effects of Bnip3 overexpression in these cells (Lee, Lee, Hanna, & Gustafsson, 2011). Finally, increased fragmentation caused by Fis1 overexpression promoted mitochondrial dysfunction and increased autophagosome formation (Gomes & Scorrano, 2008).

2.4 | Transcriptional control of mitochondrial dynamics

Transcriptional regulation is also critical for the healthy functioning of mitochondrial dynamics. The PPAR γ coactivator 1 (**PGC1**) α and β represent the main transcriptional regulators of mitochondrial biogenesis, metabolism and mitochondrial dynamics in the developing heart (Lai et al., 2008; Martin et al., 2014; Russell et al., 2004). Mice harbouring a systemic deletion of PGC1α combined with a cardiac-specific knockout of PGC1β showed mitochondrial fragmentation and elongation in the heart and developed lethal cardiomyopathy during post-natal growth, associated with altered expression of Mfn1, Opa1 and Drp1. Similarly, double systemic PGC1α and PGC1β knockout mice displayed decreased cardiac energy reserves and developed cardiac dysfunction and heart failure (Di et al., 2018). Interestingly, inducible Pgc1 α/β depletion in cardiomyocytes in adult life did not show signs of mitochondrial abnormalities and heart failure, suggesting that the essential role of this axis may be confined to the post-natal development stage (Martin et al., 2014). The direct association between PGC1α/β and gene expression of mitochondrial fusion components was supported by the observation that both PGC1α and PGC1β overexpression up-regulated Mfn1 and Mfn2 in cardiomyocytes in vitro. Specifically, PGC1α and PGC1β were found to regulate Mfn1 and Mfn2 expression, respectively, by the interaction with oestrogen-related receptor α (**ERRα**) (Liesa et al., 2008; Martin et al., 2014). PGC1α and ERRα expression were found to be down-regulated in failing human hearts (Sihag, Cresci, Li, Sucharov, & Lehman, 2009). Interestingly, mice with conditional as well as inducible cardiac overexpression of PGC1α showed myocardial mitochondrial abnormalities and developed dilated cardiomyopathy during adulthood because of unbalanced mitochondrial dynamics (Lehman et al., 2000; Russell et al., 2004).

3 | ROLE OF MITOCHONDRIAL DYNAMICS IN CARDIOVASCULAR DISEASE

Balanced mitochondrial dynamics is fundamental for maintaining cardiac structure and function both at baseline and during stress. As mitochondrial damage occurs, fusion and fission represent a flexible response to preserve a healthy mitochondrial network. In the adult heart, the process of mitochondrial dynamics is relatively low in the absence of stress as compared to the levels that can be observed in neonatal cardiomyocytes. This depends on the different distribution of mitochondria between neonatal and adult cardiomyocytes. In neonatal cardiomyocytes, mitochondria are interspersed in the cytoplasm, free to move and to undergo continuous cycles of fusion/fission. Conversely, in adult cardiomyocytes, mitochondria are confined within the perinuclear, intrafibrillar and subsarcolemmal regions, so their movements are restricted (Vasquez-Trincado et al., 2016). However, mitochondrial dynamics also occur in the adult heart, since proteins orchestrating mitochondrial dynamics are widely expressed in cardiomyocytes and giant elongated mitochondria can be observed in these cells, which indicate ongoing fusion events (Kraus & Cain, 1980; Santel et al., 2003).

3.1 | Cardiac development and baseline function

The role of mitochondrial dynamics during cardiac development and in resting state has been extensively investigated in systemic or cardiac-restricted knockout models of mitochondrial fusion and fission (Table 1). Systemic ablation of Opa1 or Mfn1/2 is embryonically lethal in murine models (Alavi et al., 2007; Chen et al., 2007). In *Drosophila*, cardiac gene silencing of Marf (analogue of the human Mfn) and Opa1 led to cardiomyopathy and impaired contractility whereas overexpression of human mitofusins (Mfn1 or Mfn2) rescued this phenotype (Dorn et al., 2011). In mice, cardiac combined deletion of Mfn1 and Mfn2 was associated with sphere-shaped, but functional mitochondria that eventually led to cardiac dysfunction by day 7 and death within 16 days after birth (Papanicolaou, Kikuchi, et al., 2012). Consistently, **tamoxifen**-induced combined ablation of Mfn1/2 in adult cardiomyocytes caused mitochondrial fragmentation and dysfunction that evolved into eccentric hypertrophy and lethal dilated cardiomyopathy (Chen et al., 2011; Song, Mihara, et al., 2015). However, mice with single cardiac deletion of Mfn1 maintained cardiac function and mitochondrial respiration, although spherical mitochondria were observed (Chen & Dorn, 2013; Papanicolaou, Ngoh, et al., 2012). In contrast, cardiac Mfn2 ablation resulted in dilated cardiomyopathy in mice (Chen & Dorn, 2013) anticipated by an early phase of mild hypertrophy (Papanicolaou et al., 2011). These mice also displayed impaired parkin-induced mitophagic clearance of damaged mitochondria and developed heart failure. This phenotype was rescued by moderate expression of catalase in the heart, which reduced mitochondrial depolarization and damage (Song et al., 2014). Notably, a marked overexpression of catalase suppressed mitophagy and failed to improve cardiac function in Mfn2 deficient mice, suggesting the existence of ROS-dependent signalling in mitochondrial clearance (Song et al., 2014). The phenotypical differences between Mfn1 and Mfn2 cardiac knockout models may be explained in different ways. First, as stated before, Mfn2 also acts as a Parkin receptor, in addition to its redundant functions with Mfn1. Secondly, Mfn2, but not Mfn1, tethers endoplasmic reticulum (ER) to mitochondria (de Brito & Scorrano, 2008). The proximity of the ER to mitochondria is essential for mitochondrial energy metabolism and calcium handling. Consequently, besides defective mitophagy, Mfn2 knockout models display

TABLE 1 Cardiac phenotype in knockout models of mitochondrial dynamics at baseline

Model	Cardiac phenotype	Cardiac mitochondrial morphology	Reference
Systemic Opa1, Mfn1, Mfn2 gene deletion	Embryonically lethal	Not applicable	(Alavi et al., 2007; Chen, McCaffery, & Chan, 2007
Cardiac Marf gene silencing (*Drosophila*)	Heart tube dilatation Dilated cardiomyopathy	Spherical Fragmented Heterogeneous	(Dorn et al., 2011
Cardiac double knockout of Mfn1-2 at midgestational and postnatal stage	Normal at birth Cardiomyopathy by post-natal day 7	Spherical Heterogeneous	(Papanicolaou et al., 2012)
Double inducible cardiac Mfn1-2 gene deletion during adulthood	Lethal dilated cardiomyopathy; eccentric hypertrophy	Fragmented	(Chen et al., 2011)
Cardiac Mfn1 deletion	Normal	Spherical, small	(Chen & Dorn, 2013; Papanicolaou et al., 2012)
Cardiac Mfn2 deletion	Dilated cardiomyopathy; hypertrophy	Enlarged	(Chen & Dorn, 2013; Papanicolaou et al., 2011)
Double cardiac deletion of Mfn1-2 at embryonic stage	Impaired cardiac development	Fragmented	(Kasahara, Cipolat, Chen, Dorn, & Scorrano, 2013)
Heterozygous Opa1 gene deletion	Normal	Enlarged	(Piquereau et al., 2012)
Cardiac deletion of Yme1l	Dilated cardiomyopathy; heart failure	Fragmented	(Wai et al., 2015)
Cardiac Drp1 deletion	Lethal heart defects	Enlarged Heterogeneous	(Ishihara et al., 2015; Kageyama et al., 2014)
Cardiac Drp1 deletion at post-natal stage	Enlarged heart	Not reported	(Song, Mihara, et al., 2015)
Tamoxifen inducible cardiac Drp1 gene deletion	Cardiac hypertrophy and fibrosis;	Elongated	(Ikeda et al., 2015; Song, Mihara, et al., 2015)
Combined Mff and Mfn1 gene deletion	Normal	Normal	(Chen et al., 2015)

Abbreviations: Drp1, dynamin-related protein 1; Marf, human analogue of Mfn; Mff, mitochondrial fission factor; Mfn1-2, mitofusin 1 and 2; Opa1, optic atrophy protein 1; Yme1l1, ATP-dependent zinc metalloprotease.

altered activation of calcium-associated pathways due to reduced mitochondrial-sarcoplasmic reticulum (SR) tethering and impaired calcium uptake (Chen et al., 2012; Konstantinidis, Lederer, Rizzuto, & Kitsis, 2012). Kasahara et al. demonstrated that gene-trapping Mfn2 and Opa1 in mouse embryonic stem cells (ESCs) impaired cardiac development and inhibited cardiomyocyte differentiation by enhancing calcium-dependent calcineurin activity, which positively modulates Notch1 signalling (Kasahara et al., 2013). However, the heterozygous cardiac-specific Opa1-defective mouse model did not show cardiac alterations in unstressed conditions and had surprisingly enlarged mitochondria. It was also observed that mitochondrial permeability transition pore (mPTP) opening was less sensitive to calcium accumulation in cardiomyocytes in vitro (Piquereau et al., 2012). Moreover, the activity of Opa1 in cardiomyocytes is regulated by the mitochondrial peptidases Yme1l and Oma1. Cardiac deletion of Yme1l resulted in Oma1 activation, which contributed to accelerated Opa1 proteolysis. The consequent abnormal mitochondrial fragmentation and metabolism ultimately culminated in dilated cardiomyopathy and heart failure (Wai et al., 2015).

Mitochondrial fission is also critical for cardiac function. Constitutive cardiomyocyte-specific Drp1-knockout mice die prematurely, showing defective mitochondrial respiration and increased accumulation of ubiquitinated proteins (Ishihara et al., 2015; Kageyama et al., 2014). Cardiac ablation of Drp1 in the immediate post-natal period also led to increased mortality (Song, Mihara, et al., 2015).

Drp1 deletion in adult cardiomyocytes determined Parkin up-regulation and consequent overactivation of mitophagy, which in turn contributed to the development of lethal cardiomyopathy. The concomitant cardiac deletion of Drp1 and Parkin improved cardiac remodelling and increased survival, highlighting a fundamental role for Parkin in the regulation of constitutive mitophagic quality control (Song, Gong, et al., 2015).

Adult mice with inducible cardiac-specific deletion of Drp1 developed dilated cardiomyopathy in unstressed conditions and died within 13 days. At the cellular level, damaged and elongated mitochondria were observed, together with decreased autophagy and increased apoptotic and necrotic cell death (Ikeda et al., 2015; Song, Mihara, et al., 2015). Similarly, systemic disruption of Mff in mice caused dilated cardiomyopathy and heart failure within the 13th week of life, showing signs of impaired mitochondrial function and increased mitophagy. This phenotype was completely rescued by concomitant deletion of Mfn1, which preserved cardiac function and lifespan (Chen et al., 2015).

Overall, these studies underline that mitochondrial dynamics is critical for the maintenance of cardiomyocyte homeostasis.

3.2 | Aging

The management of aging-associated pathophysiological alterations appears to be a prominent challenge for future decades, because of the gradual increase in life expectancy. In the heart, both histological and morphological alterations concur to the progressive development of cardiac dysfunction in the elderly (Shirakabe, Ikeda, Sciarretta, Zablocki, & Sadoshima, 2016). The aged heart is characterized by ventricular hypertrophy, diastolic dysfunction and fibrosis. Besides, unhealthy and dysfunctional mitochondria accumulate during aging, in association with increased oxidative stress. Mitochondrial permeability transition pore (mPTP) opening is also altered, leading to calcium mishandling, loss of mitochondrial membrane potential and apoptosis (Shirakabe, Ikeda, et al., 2016). Giant mitochondria with altered cristae and mtDNA damage were also observed in the aged heart, associated with imbalanced mitochondrial dynamics. Reduced expression of Mfn1/2 was observed in the aged heart of 25-month-old rats (Zhao et al., 2014). Conversely, Opa1 and Drp1 expression was increased in 36-month-old rats (Ljubicic, Menzies, & Hood, 2010).

Some evidence reported that a shift of mitochondrial dynamics towards mitochondrial fusion contributes to cell senescence, although most of these findings derived from in vitro studies. In detail, Fis1 knockdown enhanced senescence and caused elongated mitochondria in non-cardiac cell lines. Notably, the concomitant depletion of Opa1 reversed cell senescence in Fis1 knockdown cells (Lee et al., 2007). Moreover, loss of the mitochondrial E3 ubiquitin ligase MARCH5, an interacting protein of Fis1, Drp1 and Mfn2, enhanced cell senescence *in vitro* and increased Mfn1 expression levels. These effects were rescued by Drp1 overexpression (Park et al., 2010). Together, these studies suggest that promoting mitochondrial fission may reduce aging-associated alterations with mechanisms that include the recovery of mitophagy, which is usually reduced in aged tissues.

Several studies confirmed these observations *in vivo*. Transgenic mice overexpressing Opa1 developed physiological cardiac hypertrophy at 9 months of age (Varanita et al., 2015). Mice with cardiac parkin deletion developed fatal cardiomyopathy, whereas its overexpression was sufficient to delay cardiac aging in mice. Similarly, a mutation at the Pink phosphorylation site in Mfn2 suppressed cardiac mitophagy and altered cardiac metabolism (Gong et al., 2015). Similarly, p53 inhibition reduced cardiac aging-associated abnormalities by enhancing parkin-mediated mitophagy (Hoshino et al., 2013).

Impaired mitochondrial fusion may also contribute to cardiac aging. Elder heterozygous Opa1 knockout mice displayed worse cardiac function and fragmented dysfunctional mitochondria as compared to age-matched wild type mice (Chen et al., 2012). Consistently, mice with cardiac deletion of Mfn2 showed reduced left ventricular function as compared to the matched wild type at the age of 17 months (Zhao et al., 2012).

Together, these results suggest that preserving the balance between mitochondrial fusion and fission, rather than activating or inhibiting one of these mechanisms, may be the right strategy to delay cardiac abnormalities related to aging. Interestingly, a murine model in which Mfn-mediated fusion and Drp1-mediated fission were concomitantly abolished in the heart showed a better phenotype than those models that lacked fusion or fission alone, in terms of survival and development of cardiomyopathy, although impaired mitophagy and accumulation of senescent mitochondria could still be observed (Song et al., 2017).

3.3 | Cardiac hypertrophy and heart failure

Cardiac hypertrophy is a typical early adaptive response to increased cardiac workload and mechanical stress (Schiattarella & Hill, 2015; Schiattarella, Hill, & Hill, 2017). However, in cases of prolonged or chronic stress, this response may become maladaptive and ultimately lead to heart failure. Cardiomyocytes synthesize additional sarcomeres, leading to thickening of the ventricular wall and increased overall cardiac mass and size (Schiattarella et al., 2017; Schiattarella & Hill, 2015). The subcellular reorganization that underlies cardiomyocytes hypertrophy was found to require functional and responsive mitochondrial dynamics (Figure 2). In phenylephrine-stressed adult cardiomyocytes, Bnip3 was found to be up-regulated by the **forkhead box** O3a (FOXO3a) transcription factor, which contributes to mitochondrial fragmentation and apoptosis. Inhibition of FOXO3a in a rat model of heart failure with preserved ejection fraction (HFpEF) ameliorated cardiac function and preserved mitochondrial morphology and function (Chaanine et al., 2016). Likewise, a dominant-negative form of Drp1 was able to block the hypertrophic signalling induced by norepinephrine in cardiomyocyte cultures. Mechanistically, **noradrenaline** induced mitochondrial fission and dysfunction in a calcineurin/Drp1-dependent manner. In the same work, opposite results were obtained by inducing fission through Mfn2 inhibition (Pennanen et al., 2014). Coherently, in murine models of pressure overload, the Drp1 inhibitor Mdivi-1 reduced cardiac abnormal mitophagy, hypertrophy and fibrosis, without affecting BP (Givvimani et al., 2012; Hasan et al., 2018). In apparent contrast with these results, mice with constitutive cardiac-specific heterozygous deletion of *drp1* showed excessive cardiac hypertrophy and reduced cardiac function in response to transverse aortic constriction (TAC), thorough the inhibition of mitophagy (Shirakabe, Zhai, et al., 2016). These results indicate that endogenous Drp1 also plays physiological functions in response to pressure overload. Furthermore, recent work indicated that Drp1 may also act as a scaffold protein to form macromolecular protein complexes that are required for damaged mitochondrial degradation by Rab9-dependent alternative autophagy (Saito et al., 2019).

Besides fission, mitochondrial fusion was also investigated in cardiac hypertrophy and remodelling. Decreased expression of Mfn2 and Opa1 was observed in hypertrophic cardiomyocytes both *in vitro* and in rat models of heart failure (Chen, Gong, Stice, & Knowlton, 2009;

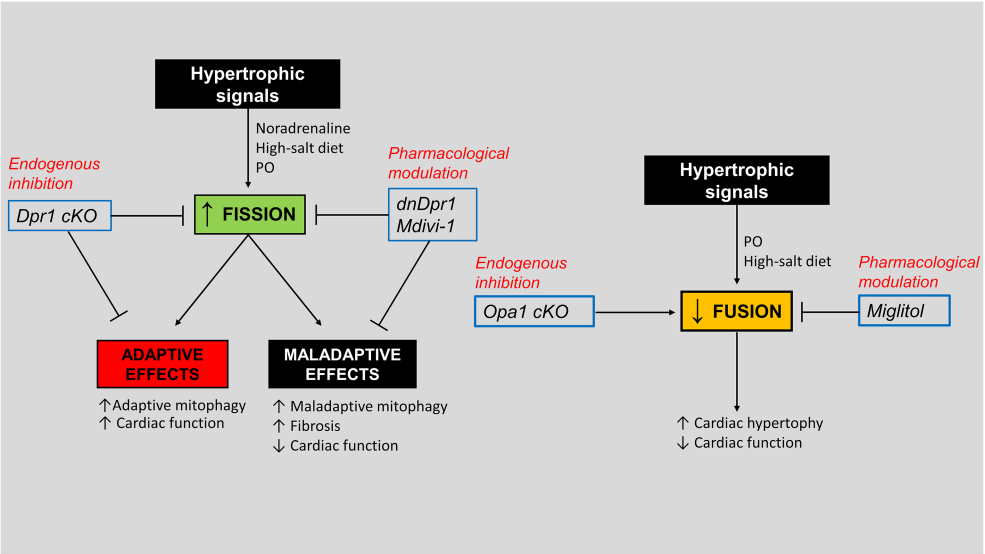

FIGURE 2 Mitochondrial dynamics in cardiac hypertrophy and remodelling. Pharmacological inhibition of mitochondrial fission during stress limits maladaptive remodelling and hypertrophy. The inhibition of endogenous Drp1 impairs adaptive mechanisms during pressure overload (left panel). Genetic inhibition of mitochondrial fusion exacerbates cardiac mass increase during pro-hypertrophic stress. The pharmacological stimulation of mitochondrial fusion reduces cardiac hypertrophy (right panel). See the text for further details. Legend: c-Het-Drp1-KO, cardiac-specific heterozygous dynamin-related protein 1 (Drp1) knockout model; c-Het-Opa1-KO, cardiac-specific heterozygous optic atrophy protein 1 (Opa1) knockout model; dnDrp1, dominant-negative Drp1; Mdivi1, Drp1 inhibitor; PO, pressure overload. The figure was made in part using tools provided by Servier Medical Arts

Fang et al., 2007). Mice with a heterozygous knockout of *opa1* subjected to 6 weeks of TAC-induced pressure overload developed a more severe form of cardiac hypertrophy as compared to matched wild type mice (Piquereau et al., 2012). Of interest, in a rat model of hypertension, the administration of **miglitol** boosted gut production of **glucagon-like peptide-1** (GLP-1) and enhanced mitochondrial fusion in the heart, improving cardiac function (Naruse et al., 2019).

3.4 | Ischaemia/reperfusion injury

Mitochondria are extremely sensitive to ischaemia/reperfusion injury (I/R) damage and increased oxidative stress, which trigger mitochondrial fission and cell death.

A large body of literature investigated mitochondrial dynamics in models of I/R (Figure 3), both *in vitro* and *in vivo*. H9c2 cardiomyocytes exposed to hypoxia/reoxygenation showed fusion inhibition, reduced ATP production and small ring-shaped (toroidal) mitochondria due to mPTP opening and potassium influx (Liu & Hajnoczky, 2011). Overexpression of Mfn2, or inhibition of Drp1, was found to reduce cell death during ischaemic stress in neurons (Jahani-Asl et al., 2007). Similarly, Mfn2 overexpression, Drp1 inhibition with mdivi-1 and dominant-negative Drp1 overexpression reduced mitochondrial fission, mPTP opening and cell death compared to control groups in HL-1 cardiomyocytes subjected to simulated I/R injury (Ong et al., 2010). Moreover, pharmacological Drp1 inhibition with mdivi-1 or P110 reduced myocardial infarct size and improved cardiac function in rodents subjected to coronary artery occlusion and reperfusion (Disatnik et al., 2013; Ong et al., 2010; Sharp et al., 2014). Similarly, adenoviral overexpression of a dominant-negative form of Drp1 enhanced protective mitochondrial uncoupling, reduced cardiac dysfunction and limited infarct size in a rat model of cardiac I/R injury (Zepeda et al., 2014). Drp1 inhibition by Pim1 exerted cardioprotective effects in the I/R injured heart by preserving mitochondrial function and reducing cell death. Mechanistically, it was observed that Pim1 overexpression impaired Drp1 mitochondrial translocation and preserved mitochondrial function in vitro (Din et al., 2013). These results would suggest that fusion promotion is beneficial in response to I/R. Consistently, a pharmacological stimulation of mitochondrial fusion before ischaemia reduced infarct size and apoptosis in rats undergoing I/R (Maneechote et al., 2019). Furthermore, Opa1 overexpressing mice were protected from I/R damage and showed sustained mitochondrial function, improved mitochondrial cristae remodelling and reduced cytochrome C release (Varanita et al., 2015).

In contrast with this evidence, heterozygous cardiac Drp1 knockout mice showed mitochondrial dysfunction, myocardial hypertrophy and elongated mitochondria in response to fastening, and exacerbated myocardial injury in response to I/R (Ikeda et al., 2015), suggesting that endogenous Drp1 also plays physiological functions in response to I/R. In this regard, mitophagy was found to be impaired in Drp1 KO mice in response to I/R (Ikeda et al., 2015). In addition, Drp1 phosphorylation at serine 616 by **RIP1** was recently found to be important for damaged mitochondria degradation in response to ischaemia by Rab9-associated autophagosomes originating from *trans* face of Golgi apparatus (Saito et al., 2019). Therefore, endogenous Drp1 may be beneficial in response to I/R injury specifically by the activation of mitophagy, which is beneficial in this condition. In this regard, Parkin

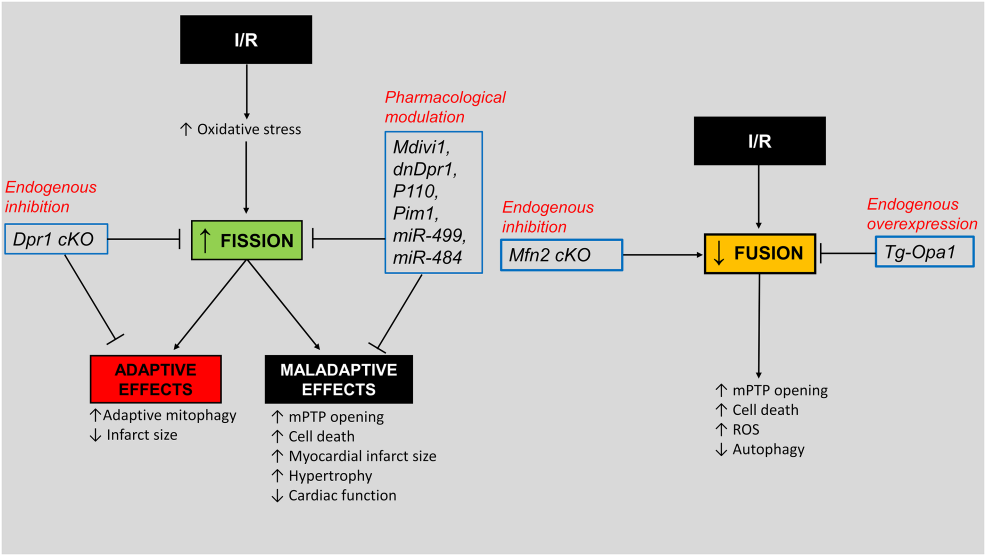

FIGURE 3 Mitochondrial dynamics and myocardial ischaemia–reperfusion. Pharmacological inhibition of mitochondrial fission during myocardial ischaemia/reperfusion (I/R) limits cardiac injury. Endogenous Drp1 reduces I/R injury by activating protective mechanisms (left panel). Mitochondrial fusion decreases during I/R. Fusion activation limits I/R injury (right panel). See the text for further details. Legend: dnDrp1, dominant-negative dynamin-related protein 1; Drp1, dynamin-related protein 1; I/R, ischaemia/reperfusion; Mdivi1, Dpr1 inhibitor; miR-499 and miR-448, microRNAs 499 and 448; Mfn1-2 cKO, cardiac-specific knockout models of mitofusin (Mfn) 1-2; mtPTP, mitochondrial permeability transition pore; Tg-Opa1, transgenic model overexpressing optic atrophy protein 1 (Opa1); P110, Drp1 inhibitor; Pim1, serine/threonine kinase. The figure was made in part using tools provided by Servier Medical Arts

knockout mice developed a larger infarct compared to control, associated with swollen and dysfunctional mitochondria, reduced mitophagy and increased Fis1 expression levels (Kubli et al., 2013).

Endogenous Mfn1/2 were also proved to be important in I/R injury, although a final consensus on the role of these proteins and mitochondrial fusion still needs to be reached. Mfn2 deficient mice subjected to I/R showed abnormal mitochondria, reduced autophagy and detrimental cardiac effects as compared to controls (Zhao et al., 2012). However, in another study, cardiac Mfn2 deletion in mice was found to improve cardiac recovery following I/R injury (Papanicolaou et al., 2011). In addition, overexpression of Mfn2 was sufficient to induce apoptosis in cardiac cells (Shen et al., 2007).

Similarly, cardiomyocytes isolated from conditional cardiac-specific Mfn1 knockout mice were resistant to ROS overload, due to a delay in mPTP opening that reduces apoptotic cell death (Papanicolaou, Ngoh, et al., 2012).

Overall, these studies suggest that both fusion and fission are fundamental for cardioprotection in response to ischaemia. Additional studies are encouraged to better understand the role of these two processes. The effects of fusion and fission may likely be dependent on the magnitude of activation of each process, on their balance and on the signalling molecules modulating them.

An attractive approach to modulate mitochondrial dynamics and reach a balance between fusion and fission is the manipulation of specific microRNAs, that is, miR-499 and miR-484. Interestingly, miR-499 overexpression prevented Drp1 mitochondrial translocation and mitochondrial fragmentation and reduced cardiac hypertrophy and dysfunction in mice subjected to I/R. Mechanistically, miR-499 overexpression repressed calcineurin, preventing its increase during cardiac ischaemia and Drp1 dephosphorylation, a modification that triggers its translocation to the mitochondria and promotes mitochondrial fission (Wang, Jiao, et al., 2011). Similarly, miR-484 overexpression suppressed mitochondrial fission and reduced infarct size by inhibiting Fis1 after I/R. This was associated with the anoxia-induced FOXO3A transcription factor, which regulates the miR-484/Fis1 pathway and increases miR-484 levels (Wang et al., 2012). Additional studies are warranted to better understand whether the cardiac effects of these miRNAs are completely attributable to their effects on mitochondrial dynamics.

3.5 | Metabolic and genetic cardiomyopathy

Cardiac abnormalities are one of the main complications of metabolic diseases, such as diabetes and obesity. Altered mitochondrial homeostasis was observed to be associated with cardiac derangements in metabolic diseases. For example, in a cardiac cell line, inhibition of mitochondrial fission rescued high-glucose (HG)-induced mitochondrial fragmentation and ROS increase (Yu, Robotham, & Yoon, 2006). Administration of the antioxidant scavenger TEMPOL reduced oxidative stress and mitochondrial fragmentation without affecting Opa1 and Drp1 expression in a murine model of type1 diabetes (T1D) (Makino, Scott, & Dillmann, 2010). Opa1 overexpression reversed mitochondrial spherification, fragmentation and dysfunction in HG-stressed neonatal cardiomyocyte cultures (Makino et al., 2011). Insulin was also found to regulate mitochondrial metabolism in

cardiomyocytes through a mechanism that depends on increased mitochondrial fusion, Opa1, Mfn2 and the Akt-mTOR-NFκB pathway (Parra et al., 2014). Similarly, Drp1 inhibition rescued mitochondrial fragmentation and cell death induced by hyperglycaemia in vitro (Yu, Sheu, Robotham, & Yoon, 2008). Gene silencing of Drp1 restored mitochondrial function and insulin signalling in an in vitro model of insulin resistance (Watanabe et al., 2014).

Notably, mice harbouring a cardiac conditional deletion of Yme1l developed cardiomyopathy and displayed mitochondrial fragmentation with preserved mitochondrial respiration, due to sustained Opa1 proteolytic activation. However, mice lacking Yme1l in both cardiac and skeletal muscles showed a healthy phenotype, despite the presence of mitochondrial fragmentation. The difference between single and double KO model relies on the cardiac switch from fatty acids to glucose catabolism in mice with single cardiac Yme11 gene deletion. On the other hand, concomitant Yme11 gene deletion in skeletal muscle causes Opa1 dysregulation and systemic glucose intolerance, thereby preserving cardiac fatty acid metabolism. In line with this evidence, feeding single cardiac-specific Yme1l knockout mice with a high-fat diet (HFD) was sufficient to prevent the development of cardiomyopathy (Wai et al., 2015). Coherently, transgenic or HFD-driven Yme1l up-regulation reduced mitochondrial fragmentation and dysfunction in a TAC model of pressure overload (Guo et al., 2018).

Fatty acid uptake may toxically overcome mitochondrial oxidative capacity and affect mitochondrial dynamics. Indeed, cardiac overexpression of long-chain acyl-CoA synthase 1 induced mitochondrial dysfunction, increased fission and promoted ROS accumulation. Mechanistically, ROS scavenging preserved mitochondrial oxidative capacity and was sufficient to reduce abnormal mitochondrial fission caused by reduced Drp1 phosphorylation and altered Opa1 processing (Tsushima et al., 2018). Interestingly, reduction of mitochondrial fission and Drp1 expression by perilipin 5 (Plin5) was sufficient to rescue cardiac dysfunction induced by lipid overload in mice harbouring mutated adipose triglyceride lipase (ATGL) (Kolleritsch et al., 2019).

Overall, alterations in mitochondrial metabolism are sufficient to dramatically compromise mitochondrial function and impair mitochondrial dynamics. Additional investigations are required to better discern cause and consequence of mitochondrial disorders observed in metabolic diseases.

The role of mitochondrial dynamics was also explored in inherited forms of cardiomyopathy. The so-named 'Python mouse' model of monogenic inherited dilative cardiomyopathy (DCM) was found to be determined by a dominant fully penetrant mutation of Drp1 gene that caused altered mitochondrial structure and function, impaired mitophagy and increased myocardial inflammation (Ashrafian et al., 2010; Cahill et al., 2015). Two rare human mutations of Mfn2 were also associated with cardiac dysfunction and mitochondrial fragmentation in *Drosophila* (Eschenbacher et al., 2012).

However, very little is still known about the association of human genetic cardiomyopathies with mitochondrial dynamics.

4 | MITOCHONDRIAL DYNAMICS IN VASCULAR DISEASES: THE EXAMPLES OF ATHEROSCLEROSIS AND STROKE

Atherosclerosis is a chronic and progressive vascular disease with a complex aetiopathogenesis. The disease is characterized by the narrowing of an artery due to the formation of a plaque enclosing inflammatory cells, oxidized-LDL and myofibroblasts (Gimbrone & Garcia-Cardena, 2016). Compelling evidence underlined a fundamental role of mitochondrial dynamics in the pathogenesis of atherosclerosis. Mfn2 expression was found to be reduced in thrombi harvested from animal models of atherosclerosis and humans (Chen et al., 2004; Liu et al., 2014). Coherently, Mfn2 overexpression was able to reduce atherosclerotic lesions in the rabbit and to inhibit neointimal formation in rat balloon-injured arteries (Chen et al., 2004; Guo et al., 2007). The role of mitochondrial dynamics in endothelial dysfunction, which is a hallmark of atherosclerosis, was studied in endothelial cells isolated from diabetic patients with reduced vascular function. These cells displayed increased expression of Fis1 and fragmented mitochondria, a phenotype that was rescued in vitro by fission inhibition (Shenouda et al., 2011). Similarly, endothelial cells treated *in vitro* with retinol-binding protein 4 (RBP4), an adipokine usually found at high levels in the blood of patients with metabolic syndrome, showed increased fission and reduced fusion (Wang et al., 2015). Besides endothelial cells, vascular smooth muscle cells (VSMCs) proliferate and migrate during the formation of the atherosclerotic plaque. VSMCs activation by platelet-derived growth factor (PDGF) was found to be associated with decreased Mfn2, while fission inhibition reduced VSMCs proliferation (Salabei & Hill, 2013). In an *ex vivo* aortic ring assay, Drp1 inhibition significantly reduced VSMCs proliferation and migration and vascular neointima formation in a model of rat carotid artery balloon injury (Lim et al., 2015). Drp1 inhibition was also found to reduce endothelial dysfunction and atherosclerosis in apolipoprotein E (ApoE) knockout diabetic mice (Wang et al., 2017) and to attenuate oxidative stress-mediated smooth muscle cell calcification (Rogers et al., 2017). Collectively, these data suggest that fission inhibition or fusion stimulation may represent a valid therapeutic strategy to slow atherosclerosis progression (Table 2).

Mitochondrial dysfunction also contributes to the pathophysiology of ischaemic stroke. In fact, restoration of mitophagy attenuated stroke predisposition in a model of essential hypertension and spontaneous stroke (Forte et al., 2019). Mitochondrial dynamics also plays a fundamental role in cell death and survival during cerebral ischaemia since mitochondrial fission was shown to be an early event that precedes neuronal loss in a murine middle cerebral artery occlusion (MCAO) I/R model (Barsoum et al., 2006). Similarly, Drp1 inhibition reduced infarct volume in MCAO models, along with improved mitochondrial function and reduced mitochondrial fragmentation (Grohm et al., 2012; Zhao, Cui, et al., 2014). Furthermore, in a permanent MCAO model, Mfn2 was found to be down-regulated (Peng et al., 2015). Considering this body of evidence, increased mitochondrial fission should be considered as a pathogenic event contributing to ischaemic stroke. Nevertheless, further studies are encouraged to

TABLE 2 Modulation of mitochondrial dynamics in atherosclerosis and ischaemic stroke

Model	Modulation of mitochondrial dynamics	Outcome	Reference
Rat balloon-injured arteries	Mfn2 overexpression	Reduction of neointima formation	(Chen et al., 2004)
Rabbits fed with an atherogenic diet	Mfn2 overexpression	Reduction of atherosclerotic lesions and VSMCs proliferation	(Guo et al., 2007)
VSMCs treated with PDGF	Pharmacological Drp1 inhibition	Reduction of VSMCs proliferation	(Salabei & Hill, 2013)
Rat carotid artery balloon injury	Pharmacological Drp1 inhibition	Reduction of vascular neointima formation	(Lim et al., 2015)
Apolipoprotein E knockout diabetic mice	Pharmacological Drp1 inhibition	Reduction of atherosclerosis and endothelial dysfunction	(Wang et al., 2017)
Calcification model of VSMCs	Pharmacological Drp1 inhibition	Reduction of calcification	(Rogers et al., 2017)
MCAO	Pharmacological Drp1 inhibition	Reduction of brain infarct volume	(Grohm et al., 2012) (Zhao, Cui, Chen, Dong, & Liu, 2014)

Abbreviations: Drp1, dynamin-related protein 1; Mfn2, mitofusin 2; MCAO, middle cerebral artery occlusion; VSMCs, vascular smooth muscle cells.

define the role of mitochondrial dynamics in cerebral vascular cells and neuronal death.

5 | MITOCHONDRIAL DYNAMICS IN HUMAN STUDIES

To date, most of the studies in humans remain observational and limited to ultrastructural characterization and gene expression profiling of cardiac biopsies. For example, giant mitochondria were observed in mitochondrial cardiomyopathy. Although the cause responsible for the formation of abnormal structures needs to be fully elucidated, it was proposed that adjacent mitochondria fuse as a compensatory mechanism to mtDNA mutations (Hoppel, Tandler, Fujioka, & Riva, 2009; Kanzaki et al., 2010). Smaller mitochondria associated with low levels of Mfn2 and Opa1 were observed in skeletal muscle of diabetic and obese subjects (Kelley, He, Menshikova, & Ritov, 2002; Zorzano, Liesa, & Palacin, 2009). Interestingly, in explanted failing human hearts, Opa1 protein levels were found to be down-regulated whereas its mRNA was unaffected, suggesting the activity of a post-translational regulatory mechanism (Chen et al., 2009). In a recent study, mitochondrial fragmentation was reported in the myocardium of patients with heart failure with both preserved or reduced ejection fraction, along with an increased expression of Bnip3 and Drp1 (Chaanine et al., 2019). Remarkably, samples collected from diabetic patients before the onset of cardiomyopathy showed mitochondrial dysfunction, impaired contraction and mitochondrial fragmentation, in association with decreased expression of Mfn1 (Montaigne et al., 2014). As previously reviewed, the causative relationship between mutations in genes coding for mitochondrial dynamics components has been defined in severe inherited neurological and neurodegenerative disorders (e.g. Charcot Marie Tooth syndrome). It should be taken into consideration that patients affected by these pathologies do not display relevant spontaneous cardiac abnormalities in most cases. However, Spiegel et al. reported a fatal infantile-onset encephalopathy associated with progressive hypertrophic cardiomyopathy in two sisters carrying a homozygous mutation in *opa1* (Spiegel et al., 2016). Lastly, it is worth mentioning that genome-wide association studies (GWAS) identified a correlation between the presence of a variant in *opa1* (rs528908640) and diastolic BP level (Nagy et al., 2017). Similarly, a different polymorphism in *opa1* was found to be associated with age-dependent hypertension (Jin et al., 2011). In conclusion, mitochondrial dynamics appears to be involved in human pathology, although a limited number of studies was conducted to understand the impact of its dysregulation on the development of cardiovascular diseases in human patients.

6 | PHARMACOLOGICAL MODULATION OF MITOCHONDRIAL DYNAMICS

Mitochondria are crucial targets for the treatment of cardiovascular diseases. In the last two decades, efforts have been done to develop therapeutic strategies for the improvement of mitochondrial function. To date, many different approaches showed promising results in preclinical and clinical studies. One strategy consists in attenuating mitochondrial oxidative stress, which generally increases during cardiovascular stress. Mitochondria-targeted antioxidants (i.e. MitoQ) were shown to reduce doxorubicin-induced cardiomyopathy and to improve endothelial function in healthy elderly individuals (Chandran et al., 2009; Rossman et al., 2018). To date, a further study is still recruiting participants to assess the effect of MitoQ supplementation on cardiac and vascular function (NCT03586414). **Nicotinamide adenine dinucleotide** (NAD^+) supplementation represents another potential strategy to recover mitochondrial function since nicotinamide riboside and nicotinamide mononucleotide were found to improve, respectively, cardiac function and to reduce stroke occurrence in different preclinical models (Diguet et al., 2018; Forte, Bianchi, et al., 2019). Ongoing clinical trials are testing the effects of NAD^+

supplementation in patients suffering from heart failure (NCT03423342, NCT03727646 and NCT03565328). Among natural products, **resveratrol** and **curcumin** have been proven to improve mitochondrial function in animal models of cardiovascular diseases (Forte, Palmerio, et al., 2019). Moreover, caloric restriction and exercise represent appropriate lifestyle interventions able to rescue mitochondrial dysfunction (Dai, Rabinovitch, & Ungvari, 2012). However, these approaches are aimed to preserve mitochondrial health only in general terms. More specifically, homeostatic mitochondrial dynamics and cardiac function were rescued in a canine model of heart failure with the chronic administration of the cell-permeable small peptide Szeto-Schiller SS-31 (also named 'elamipretide'), which normalized the increased levels of fission-associated proteins and the decreased levels of the fusion-associated ones (Sabbah, Gupta, Singh-Gupta, Zhang, & Lanfear, 2018). To date, SS-31 is being used in clinical trials in subjects with heart failure (NCT02914665, NCT02914665, NCT02388464, NCT02814097 and NCT02788747).

Notably, specific agents able to target mitochondrial dynamics proteins have also been developed. As discussed before, mdivi-1 and P110 represent two fission inhibitors that showed cardiac protective effects in various animal models. However, whether mdivi-1 or P110 effects are attributable only to fission inhibition is still an object of debate (Bonora et al., 2019). Dynasore, a small non-competitive dynamin GTPase inhibitor, is another potential Drp1 inhibitor that has been found to reduce myocardial death in isolated mouse hearts subjected to I/R (Gao et al., 2013). However, since dynasore acts on multiple targets, it is particularly difficult to determine the correct dose to specifically target mitochondrial fission. Intriguingly, Franco et al. developed a cell-permeant small peptide that restores the functional conformation of a mutant Mfn2 and stimulates mitochondrial fusion (Franco et al., 2016). However, it is worth stressing that modulating mitochondrial dynamics may be beneficial only in the short term since prolonged inhibition of fission is likely to exert detrimental cardiac effects in the long term. This particular aspect is critical for the development of new therapies and, to the best of our knowledge, modulators of mitochondrial dynamics have not been tested on patients affected by cardiovascular diseases yet.

7 | CONCLUSIONS AND PERSPECTIVES

An increasing body of literature defines a crucial role for mitochondrial dynamics in cardiac homeostasis. Alterations in key components of mitochondrial fusion or fission in vivo revealed that the balance of these processes is critical in both basal and stressed conditions. Moreover, modulating fission and fusion is emerging as a valid therapeutic strategy in virtually all cardiovascular diseases that are associated with disorders in mitochondrial dynamics. However, further studies will be needed to improve our understanding of these processes and to define the best tuning for protecting the heart during stress. This will be important for developing promising pharmacological agents for clinical translation. Most of the studies described in this article were conducted in transgenic animals, and little is known about pharmacological modulation of mitochondrial dynamics. Nevertheless, the authors who used pharmacological tools for this purpose showed solid and encouraging results. A better evaluation of mitochondrial dynamics in human samples may represent an important step towards the clinical translation of the reviewed findings. Coupling biopsies analyses with indirect markers of mitochondrial dynamics will help clinicians to improve risk stratification of patients with CVDs and follow the response to new therapies. In order to be successful in this task, it will also be important to conceive new tools for a more accurate and easier assessment of mitochondrial dynamics and turnover.

7.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2017), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID


Pietro Ameri https://orcid.org/0000-0001-7167-7287

REFERENCES

Alavi, M. V., Bette, S., Schimpf, S., Schuettauf, F., Schraermeyer, U., Wehrl, H. F., … Knipper, M. (2007). A splice site mutation in the murine Opa1 gene features pathology of autosomal dominant optic atrophy. *Brain*, *130*, 1029–1042.

Alexander, S. P. H., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., … Southan, C. (2019). The Concise Guide to pharmacology 2019/20: Introduction and other protein targets. *British Journal of Pharmacology*, *176*(Suppl 1), S1–S20.

Anand, R., Wai, T., Baker, M. J., Kladt, N., Schauss, A. C., Rugarli, E., & Langer, T. (2014). The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. *The Journal of Cell Biology*, *204*, 919–929. https://doi.org/10.1083/jcb.201308006

Ashrafian, H., Docherty, L., Leo, V., Towlson, C., Neilan, M., Steeples, V., … Dear, T. N. (2010). A mutation in the mitochondrial fission gene Dnm1l leads to cardiomyopathy. *PLoS Genetics*, *6*, e1001000. https://doi.org/10.1371/journal.pgen.1001000

Barsoum, M. J., Yuan, H., Gerencser, A. A., Liot, G., Kushnareva, Y., Graber, S., … White, A. D. (2006). Nitric oxide-induced mitochondrial fission is regulated by dynamin-related GTPases in neurons. *The EMBO Journal*, *25*, 3900–3911. https://doi.org/10.1038/sj.emboj.7601253

Basu, K., Lajoie, D., Aumentado-Armstrong, T., Chen, J., Koning, R. I., Bossy, B., … Rouiller, I. (2017). Molecular mechanism of DRP1 assembly studied in vitro by cryo-electron microscopy. *PLoS ONE*, *12*, e0179397. https://doi.org/10.1371/journal.pone.0179397

Bhandari, P., Song, M., Chen, Y., Burelle, Y., & Dorn, G. W. 2nd (2014). Mitochondrial contagion induced by Parkin deficiency in Drosophila hearts and its containment by suppressing mitofusin. *Circulation Research*, *114*, 257–265. https://doi.org/10.1161/CIRCRESAHA.114.302734

Bonneau, D., Colin, E., Oca, F., Ferre, M., Chevrollier, A., Gueguen, N., … Rio, M. (2014). Early-onset Behr syndrome due to compound heterozygous mutations in OPA1. *Brain*, *137*, e301. https://doi.org/10.1093/brain/awu184

Bonora, M., Wieckowski, M. R., Sinclair, D. A., Kroemer, G., Pinton, P., & Galluzzi, L. (2019). Targeting mitochondria for cardiovascular disorders: Therapeutic potential and obstacles. *Nature Reviews. Cardiology*, *16*, 33–55.

Bossy-Wetzel, E., Barsoum, M. J., Godzik, A., Schwarzenbacher, R., & Lipton, S. A. (2003). Mitochondrial fission in apoptosis, neurodegeneration and aging. *Current Opinion in Cell Biology*, *15*, 706–716.

Burman, J. L., Pickles, S., Wang, C., Sekine, S., Vargas, J. N. S., Zhang, Z., … Youle, R. J. (2017). Mitochondrial fission facilitates the selective mitophagy of protein aggregates. *The Journal of Cell Biology*, *216*, 3231–3247. https://doi.org/10.1083/jcb.201612106

Cahill, T. J., Leo, V., Kelly, M., Stockenhuber, A., Kennedy, N. W., Bao, L., … Ashrafian, H. (2015). Resistance of dynamin-related protein 1 oligomers to disassembly impairs mitophagy, resulting in myocardial inflammation and heart failure. *The Journal of Biological Chemistry*, *290*, 25,907–25,919. https://doi.org/10.1074/jbc.M115.665695

Chaanine, A. H., Joyce, L. D., Stulak, J. M., Maltais, S., Joyce, D. L., Dearani, J. A., … Redfield, M. M. (2019). Mitochondrial morphology, dynamics, and function in human pressure overload or ischemic heart disease with preserved or reduced ejection fraction. *Circulation. Heart Failure*, *12*, e005131.

Chaanine, A. H., Kohlbrenner, E., Gamb, S. I., Guenzel, A. J., Klaus, K., Fayyaz, A. U., … Redfield, M. M. (2016). FOXO3a regulates BNIP3 and modulates mitochondrial calcium, dynamics, and function in cardiac stress. *American Journal of Physiology. Heart and Circulatory Physiology*, *311*, H1540–H1559. https://doi.org/10.1152/ajpheart.00549.2016

Chan, D. C. (2006). Mitochondrial fusion and fission in mammals. *Annual Review of Cell and Developmental Biology*, *22*, 79–99.

Chandran, K., Aggarwal, D., Migrino, R. Q., Joseph, J., McAllister, D., Konorev, E. A., … Kalyanaraman, B. (2009). Doxorubicin inactivates myocardial cytochrome c oxidase in rats: Cardioprotection by Mito-Q. *Biophysical Journal*, *96*, 1388–1398. https://doi.org/10.1016/j.bpj.2008.10.042

Chang, C. R., & Blackstone, C. (2007a). Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology. *The Journal of Biological Chemistry*, *282*, 21,583–21,587.

Chang, C. R., & Blackstone, C. (2007b). Drp1 phosphorylation and mitochondrial regulation. *EMBO Reports*, *8*, 1088–1089. author reply 1089–1090

Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., & Chan, D. C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *The Journal of Cell Biology*, *160*, 189–200.

Chen, H., McCaffery, J. M., & Chan, D. C. (2007). Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell*, *130*, 548–562.

Chen, H., Ren, S., Clish, C., Jain, M., Mootha, V., McCaffery, J. M., & Chan, D. C. (2015). Titration of mitochondrial fusion rescues Mff-deficient cardiomyopathy. *The Journal of Cell Biology*, *211*, 795–805. https://doi.org/10.1083/jcb.201507035

Chen, K. H., Guo, X., Ma, D., Guo, Y., Li, Q., Yang, D., … Tang, J. (2004). Dysregulation of HSG triggers vascular proliferative disorders. *Nature Cell Biology*, *6*, 872–883. https://doi.org/10.1038/ncb1161

Chen, L., Gong, Q., Stice, J. P., & Knowlton, A. A. (2009). Mitochondrial OPA1, apoptosis, and heart failure. *Cardiovascular Research*, *84*, 91–99.

Chen, L., Liu, T., Tran, A., Lu, X., Tomilov, A. A., Davies, V., … Knowlton, A. A. (2012). OPA1 mutation and late-onset cardiomyopathy: Mitochondrial dysfunction and mtDNA instability. *Journal of the American Heart Association*, *1*, e003012.

Chen, M., Chen, Z., Wang, Y., Tan, Z., Zhu, C., Li, Y., … Chen, Q. (2016). Mitophagy receptor FUNDC1 regulates mitochondrial dynamics and mitophagy. *Autophagy*, *12*, 689–702. https://doi.org/10.1080/15548627.2016.1151580

Chen, Y., Csordas, G., Jowdy, C., Schneider, T. G., Csordas, N., Wang, W., … Nerbonne, J. M. (2012). Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle Ca(2+) crosstalk. *Circulation Research*, *111*, 863–875. https://doi.org/10.1161/CIRCRESAHA.112.266585

Chen, Y., & Dorn, G. W. 2nd (2013). PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science*, *340*, 471–475.

Chen, Y., Liu, Y., & Dorn, G. W. 2nd (2011). Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circulation Research*, *109*, 1327–1331.

Cipolat, S., Martins de Brito, O., Dal Zilio, B., & Scorrano, L. (2004). OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 15,927–15,932.

Dai, D. F., Rabinovitch, P. S., & Ungvari, Z. (2012). Mitochondria and cardiovascular aging. *Circulation Research*, *110*, 1109–1124.

Davies, V. J., Hollins, A. J., Piechota, M. J., Yip, W., Davies, J. R., White, K. E., … Votruba, M. (2007). Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. *Human Molecular Genetics*, *16*, 1307–1318. https://doi.org/10.1093/hmg/ddm079

de Brito, O. M., & Scorrano, L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature*, *456*, 605–610.

Di, W., Lv, J., Jiang, S., Lu, C., Yang, Z., Ma, Z., … Xu, B. (2018). PGC-1: The energetic regulator in cardiac metabolism. *Current Issues in Molecular Biology*, *28*, 29–46. https://doi.org/10.21775/cimb.028.029

Diguet, N., Trammell, S. A. J., Tannous, C., Deloux, R., Piquereau, J., Mougenot, N., … Mericskay, M. (2018). Nicotinamide riboside preserves cardiac function in a mouse model of dilated cardiomyopathy. *Circulation*, *137*, 2256–2273. https://doi.org/10.1161/CIRCULATIONAHA.116.026099

Din, S., Mason, M., Volkers, M., Johnson, B., Cottage, C. T., Wang, Z., … Sussman, M. A. (2013). Pim-1 preserves mitochondrial morphology by inhibiting dynamin-related protein 1 translocation. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 5969–5974. https://doi.org/10.1073/pnas.1213294110

Disatnik, M. H., Ferreira, J. C., Campos, J. C., Gomes, K. S., Dourado, P. M., Qi, X., & Mochly-Rosen, D. (2013). Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. *Journal of the American Heart Association*, *2*, e000461.

Dorn, G. W. 2nd, Clark, C. F., Eschenbacher, W. H., Kang, M. Y., Engelhard, J. T., Warner, S. J., … Jowdy, C. C. (2011). MARF and Opa1 control mitochondrial and cardiac function in Drosophila. *Circulation Research*, *108*, 12–17. https://doi.org/10.1161/CIRCRESAHA.110.236745

Durcan, T. M., Tang, M. Y., Perusse, J. R., Dashti, E. A., Aguileta, M. A., McLelland, G. L., … Coulombe, B. (2014). USP8 regulates mitophagy by removing K6-linked ubiquitin conjugates from parkin. *The EMBO Journal*, *33*, 2473–2491. https://doi.org/10.15252/embj.201489729

Eschenbacher, W. H., Song, M., Chen, Y., Bhandari, P., Zhao, P., Jowdy, C. C., … Dorn, G. W. (2012). Two rare human mitofusin 2 mutations alter mitochondrial dynamics and induce retinal and cardiac pathology in Drosophila. *PLoS ONE*, *7*, e44296. https://doi.org/10.1371/journal.pone.0044296

Fang, L., Moore, X. L., Gao, X. M., Dart, A. M., Lim, Y. L., & Du, X. J. (2007). Down-regulation of mitofusin-2 expression in cardiac hypertrophy in vitro and in vivo. *Life Sciences*, *80*, 2154–2160.

Filadi, R., Pendin, D., & Pizzo, P. (2018). Mitofusin 2: From functions to disease. *Cell Death & Disease*, *9*, 330.

Forte, M., Bianchi, F., Cotugno, M., Marchitti, S., De Falco, E., Raffa, S., … Pagano, F. (2019). Pharmacological restoration of autophagy reduces hypertension-related stroke occurrence. *Autophagy*, 1–14. https://doi.org/10.1080/15548627.2019.1687215

Forte, M., Palmerio, S., Bianchi, F., Volpe, M., & Rubattu, S. (2019). Mitochondrial complex I deficiency and cardiovascular diseases: Current evidence and future directions. *Journal of Molecular Medicine (Berlin, Germany)*, *97*, 579–591.

Franco, A., Kitsis, R. N., Fleischer, J. A., Gavathiotis, E., Kornfeld, O. S., Gong, G., … Dorn, G. W. II (2016). Correcting mitochondrial fusion by manipulating mitofusin conformations. *Nature*, *540*, 74–79. https://doi.org/10.1038/nature20156

Gao, D., Zhang, L., Dhillon, R., Hong, T. T., Shaw, R. M., & Zhu, J. (2013). Dynasore protects mitochondria and improves cardiac lusitropy in Langendorff perfused mouse heart. *PLoS ONE*, *8*, e60967.

Gimbrone, M. A. Jr., & Garcia-Cardena, G. (2016). Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circulation Research*, *118*, 620–636.

Givvimani, S., Munjal, C., Tyagi, N., Sen, U., Metreveli, N., & Tyagi, S. C. (2012). Mitochondrial division/mitophagy inhibitor (Mdivi) ameliorates pressure overload induced heart failure. *PLoS ONE*, *7*, e32388.

Gomes, L. C., & Scorrano, L. (2008). High levels of Fis1, a pro-fission mitochondrial protein, trigger autophagy. *Biochimica et Biophysica Acta*, *1777*, 860–866.

Gong, G., Song, M., Csordas, G., Kelly, D. P., Matkovich, S. J., & Dorn, G. W. 2nd (2015). Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice. *Science*, *350*, aad2459. https://doi.org/10.1126/science.aad2459

Grohm, J., Kim, S. W., Mamrak, U., Tobaben, S., Cassidy-Stone, A., Nunnari, J., … Culmsee, C. (2012). Inhibition of Drp1 provides neuroprotection in vitro and in vivo. *Cell Death and Differentiation*, *19*, 1446–1458. https://doi.org/10.1038/cdd.2012.18

Guo, C., Hildick, K. L., Luo, J., Dearden, L., Wilkinson, K. A., & Henley, J. M. (2013). SENP3-mediated deSUMOylation of dynamin-related protein 1 promotes cell death following ischaemia. *The EMBO Journal*, *32*, 1514–1528.

Guo, Y., Wang, Z., Qin, X., Xu, J., Hou, Z., Yang, H., … Gao, F. (2018). Enhancing fatty acid utilization ameliorates mitochondrial fragmentation and cardiac dysfunction via rebalancing optic atrophy 1 processing in the failing heart. *Cardiovascular Research*, *114*, 979–991. https://doi.org/10.1093/cvr/cvy052

Guo, Y. H., Chen, K., Gao, W., Li, Q., Chen, L., Wang, G. S., & Tang, J. (2007). Overexpression of Mitofusin 2 inhibited oxidized low-density lipoprotein induced vascular smooth muscle cell proliferation and reduced atherosclerotic lesion formation in rabbit. *Biochemical and Biophysical Research Communications*, *363*, 411–417. https://doi.org/10.1016/j.bbrc.2007.08.191

Harding, S. D., Sharman, J. L., Faccenda, E., Southan, C., Pawson, A. J., Ireland, S., … Bryant, C. (2017). The IUPHAR/BPS Guide to pharmacology in 2018: Updates and expansion to encompass the new guide to immunopharmacology. *Nucleic Acids Research*, *46*, D1091–D1106.

Hasan, P., Saotome, M., Ikoma, T., Iguchi, K., Kawasaki, H., Iwashita, T., … Maekawa, Y. (2018). Mitochondrial fission protein, dynamin-related protein 1, contributes to the promotion of hypertensive cardiac hypertrophy and fibrosis in Dahl-salt sensitive rats. *Journal of Molecular and Cellular Cardiology*, *121*, 103–106. https://doi.org/10.1016/j.yjmcc.2018.07.004

Hoppel, C. L., Tandler, B., Fujioka, H., & Riva, A. (2009). Dynamic organization of mitochondria in human heart and in myocardial disease. *The International Journal of Biochemistry & Cell Biology*, *41*, 1949–1956.

Hoshino, A., Mita, Y., Okawa, Y., Ariyoshi, M., Iwai-Kanai, E., Ueyama, T., … Matoba, S. (2013). Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. *Nature Communications*, *4*, 2308. https://doi.org/10.1038/ncomms3308

Ikeda, Y., Shirakabe, A., Maejima, Y., Zhai, P., Sciarretta, S., Toli, J., … Sadoshima, J. (2015). Endogenous Drp1 mediates mitochondrial autophagy and protects the heart against energy stress. *Circulation Research*, *116*, 264–278. https://doi.org/10.1161/CIRCRESAHA.116.303356

Ishihara, T., Ban-Ishihara, R., Maeda, M., Matsunaga, Y., Ichimura, A., Kyogoku, S., … Ishihara, N. (2015). Dynamics of mitochondrial DNA nucleoids regulated by mitochondrial fission is essential for maintenance of homogeneously active mitochondria during neonatal heart development. *Molecular and Cellular Biology*, *35*, 211–223. https://doi.org/10.1128/MCB.01054-14

Jahani-Asl, A., Cheung, E. C., Neuspiel, M., MacLaurin, J. G., Fortin, A., Park, D. S., … Slack, R. S. (2007). Mitofusin 2 protects cerebellar granule neurons against injury-induced cell death. *The Journal of Biological Chemistry*, *282*, 23,788–23,798. https://doi.org/10.1074/jbc.M703812200

Jin, H. S., Sober, S., Hong, K. W., Org, E., Kim, B. Y., Laan, M., … Jeong, S. Y. (2011). Age-dependent association of the polymorphisms in the mitochondria-shaping gene, OPA1, with blood pressure and hypertension in Korean population. *American Journal of Hypertension*, *24*, 1127–1135. https://doi.org/10.1038/ajh.2011.131

Kageyama, Y., Hoshijima, M., Seo, K., Bedja, D., Sysa-Shah, P., Andrabi, S. A., … Sesaki, H. (2014). Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain. *The EMBO Journal*, *33*, 2798–2813. https://doi.org/10.15252/embj.201488658

Kanzaki, Y., Terasaki, F., Okabe, M., Otsuka, K., Katashima, T., Fujita, S., … Kitaura, Y. (2010). Giant mitochondria in the myocardium of a patient with mitochondrial cardiomyopathy: Transmission and 3-dimensional scanning electron microscopy. *Circulation*, *121*, 831–832. https://doi.org/10.1161/CIR.0b013e3181d22e2d

Karbowski, M., Lee, Y. J., Gaume, B., Jeong, S. Y., Frank, S., Nechushtan, A., … Youle, R. J. (2002). Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. *The Journal of Cell Biology*, *159*, 931–938. https://doi.org/10.1083/jcb.200209124

Kasahara, A., Cipolat, S., Chen, Y., Dorn, G. W. 2nd, & Scorrano, L. (2013). Mitochondrial fusion directs cardiomyocyte differentiation via calcineurin and Notch signaling. *Science*, *342*, 734–737.

Kelley, D. E., He, J., Menshikova, E. V., & Ritov, V. B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes*, *51*, 2944–2950.

Kim, E. Y., Zhang, Y., Beketaev, I., Segura, A. M., Yu, W., Xi, Y., … Wang, J. (2015). SENP5, a SUMO isopeptidase, induces apoptosis and cardiomyopathy. *Journal of Molecular and Cellular Cardiology*, *78*, 154–164. https://doi.org/10.1016/j.yjmcc.2014.08.003

Kim, H., Scimia, M. C., Wilkinson, D., Trelles, R. D., Wood, M. R., Bowtell, D., … Ronai, Z.'. A. (2011). Fine-tuning of Drp1/Fis1 availability by AKAP121/Siah2 regulates mitochondrial adaptation to hypoxia. *Molecular Cell*, *44*, 532–544. https://doi.org/10.1016/j.molcel.2011.08.045

Kolleritsch, S., Kien, B., Schoiswohl, G., Diwoky, C., Schreiber, R., Heier, C., … Haemmerle, G. (2019). Low cardiac lipolysis reduces mitochondrial fission and prevents lipotoxic heart dysfunction in Perilipin 5 mutant mice. *Cardiovascular Research*. https://doi.org/10.1093/cvr/cvz119

Konstantinidis, K., Lederer, W. J., Rizzuto, R., & Kitsis, R. N. (2012). Mitofusin 2 joins the sarcoplasmic reticulum and mitochondria at the hip to sustain cardiac energetics. *Circulation Research*, *111*, 821–823.

Kraus, B., & Cain, H. (1980). Giant mitochondria in the human myocardium—Morphogenesis and fate. *Virchows Archiv. B, Cell Pathology Including Molecular Pathology*, *33*, 77–89.

Kubli, D. A., Zhang, X., Lee, Y., Hanna, R. A., Quinsay, M. N., Nguyen, C. K., … Gustafsson, Å. B. (2013). Parkin protein deficiency exacerbates

cardiac injury and reduces survival following myocardial infarction. *The Journal of Biological Chemistry*, *288*, 915–926. https://doi.org/10.1074/jbc.M112.411363

Labrousse, A. M., Zappaterra, M. D., Rube, D. A., & van der Bliek, A. M. (1999). *C. elegans* dynamin-related protein DRP-1 controls severing of the mitochondrial outer membrane. *Molecular Cell*, *4*, 815–826.

Lai, L., Leone, T. C., Zechner, C., Schaeffer, P. J., Kelly, S. M., Flanagan, D. P., … Kelly, D. P. (2008). Transcriptional coactivators PGC-1α and PGC-lβ control overlapping programs required for perinatal maturation of the heart. *Genes & Development*, *22*, 1948–1961. https://doi.org/10.1101/gad.1661708

Lee, S., Jeong, S. Y., Lim, W. C., Kim, S., Park, Y. Y., Sun, X., … Cho, H. (2007). Mitochondrial fission and fusion mediators, hFis1 and OPA1, modulate cellular senescence. *The Journal of Biological Chemistry*, *282*, 22,977–22,983. https://doi.org/10.1074/jbc.M700679200

Lee, Y., Lee, H. Y., Hanna, R. A., & Gustafsson, A. B. (2011). Mitochondrial autophagy by Bnip3 involves Drp1-mediated mitochondrial fission and recruitment of Parkin in cardiac myocytes. *American Journal of Physiology. Heart and Circulatory Physiology*, *301*, H1924–H1931.

Lehman, J. J., Barger, P. M., Kovacs, A., Saffitz, J. E., Medeiros, D. M., & Kelly, D. P. (2000). Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. *The Journal of Clinical Investigation*, *106*, 847–856.

Liesa, M., Borda-d'Agua, B., Medina-Gomez, G., Lelliott, C. J., Paz, J. C., Rojo, M., … Zorzano, A. (2008). Mitochondrial fusion is increased by the nuclear coactivator PGC-1β. *PLoS ONE*, *3*, e3613. https://doi.org/10.1371/journal.pone.0003613

Lim, S., Lee, S. Y., Seo, H. H., Ham, O., Lee, C., Park, J. H., … Hwang, K. C. (2015). Regulation of mitochondrial morphology by positive feedback interaction between PKCδ and Drp1 in vascular smooth muscle cell. *Journal of Cellular Biochemistry*, *116*, 648–660. https://doi.org/10.1002/jcb.25016

Liu, C., Ge, B., He, C., Zhang, Y., Liu, X., Liu, K., … Guo, X. (2014). Mitofusin 2 decreases intracellular lipids in macrophages by regulating peroxisome proliferator-activated receptor-γ. *Biochemical and Biophysical Research Communications*, *450*, 500–506. https://doi.org/10.1016/j.bbrc.2014.06.005

Liu, X., & Hajnoczky, G. (2011). Altered fusion dynamics underlie unique morphological changes in mitochondria during hypoxia-reoxygenation stress. *Cell Death and Differentiation*, *18*, 1561–1572.

Ljubicic, V., Menzies, K. J., & Hood, D. A. (2010). Mitochondrial dysfunction is associated with a pro-apoptotic cellular environment in senescent cardiac muscle. *Mechanisms of Ageing and Development*, *131*, 79–88.

Loson, O. C., Song, Z., Chen, H., & Chan, D. C. (2013). Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. *Molecular Biology of the Cell*, *24*, 659–667.

Lutz, A. K., Exner, N., Fett, M. E., Schlehe, J. S., Kloos, K., Lammermann, K., … Bouman, L. (2009). Loss of parkin or PINK1 function increases Drp1-dependent mitochondrial fragmentation. *The Journal of Biological Chemistry*, *284*, 22938–22951. https://doi.org/10.1074/jbc.M109.035774

MacVicar, T., & Langer, T. (2016). OPA1 processing in cell death and disease—The long and short of it. *Journal of Cell Science*, *129*, 2297–2306.

Makino, A., Scott, B. T., & Dillmann, W. H. (2010). Mitochondrial fragmentation and superoxide anion production in coronary endothelial cells from a mouse model of type 1 diabetes. *Diabetologia*, *53*, 1783–1794.

Makino, A., Suarez, J., Gawlowski, T., Han, W., Wang, H., Scott, B. T., & Dillmann, W. H. (2011). Regulation of mitochondrial morphology and function by O-GlcNAcylation in neonatal cardiac myocytes. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *300*, R1296–R1302. https://doi.org/10.1152/ajpregu.00437.2010

Maneechote, C., Palee, S., Kerdphoo, S., Jaiwongkam, T., Chattipakorn, S. C., & Chattipakorn, N. (2019). Balancing mitochondrial dynamics via increasing mitochondrial fusion attenuates infarct size and left ventricular dysfunction in rats with cardiac ischemia/-reperfusion injury. *Clinical Science (London, England)*, *133*, 497–513.

Martin, O. J., Lai, L., Soundarapandian, M. M., Leone, T. C., Zorzano, A., Keller, M. P., … Kelly, D. P. (2014). A role for peroxisome proliferator-activated receptor γ coactivator-1 in the control of mitochondrial dynamics during postnatal cardiac growth. *Circulation Research*, *114*, 626–636. https://doi.org/10.1161/CIRCRESAHA.114.302562

Montaigne, D., Marechal, X., Coisne, A., Debry, N., Modine, T., Fayad, G., … Staels, B. (2014). Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation*, *130*, 554–564. https://doi.org/10.1161/CIRCULATIONAHA.113.008476

Nagy, R., Boutin, T. S., Marten, J., Huffman, J. E., Kerr, S. M., Campbell, A., … Hayward, C. (2017). Exploration of haplotype research consortium imputation for genome-wide association studies in 20,032 Generation Scotland participants. *Genome Medicine*, *9*, 23. https://doi.org/10.1186/s13073-017-0414-4

Naruse, G., Kanamori, H., Yoshida, A., Minatoguchi, S., Kawaguchi, T., Iwasa, M., … Minatoguchi, S. (2019). The intestine responds to heart failure by enhanced mitochondrial fusion through glucagon-like peptide-1 signalling. *Cardiovascular Research*, *115*, 1873–1885. https://doi.org/10.1093/cvr/cvz002

Ong, S. B., Subrayan, S., Lim, S. Y., Yellon, D. M., Davidson, S. M., & Hausenloy, D. J. (2010). Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. *Circulation*, *121*, 2012–2022.

Papanicolaou, K. N., Khairallah, R. J., Ngoh, G. A., Chikando, A., Luptak, I., O'Shea, K. M., … Walsh, K. (2011). Mitofusin-2 maintains mitochondrial structure and contributes to stress-induced permeability transition in cardiac myocytes. *Molecular and Cellular Biology*, *31*, 1309–1328. https://doi.org/10.1128/MCB.00911-10

Papanicolaou, K. N., Kikuchi, R., Ngoh, G. A., Coughlan, K. A., Dominguez, I., Stanley, W. C., & Walsh, K. (2012). Mitofusins 1 and 2 are essential for postnatal metabolic remodeling in heart. *Circulation Research*, *111*, 1012–1026. https://doi.org/10.1161/CIRCRESAHA.112.274142

Papanicolaou, K. N., Ngoh, G. A., Dabkowski, E. R., O'Connell, K. A., Ribeiro, R. F. Jr., Stanley, W. C., & Walsh, K. (2012). Cardiomyocyte deletion of mitofusin-1 leads to mitochondrial fragmentation and improves tolerance to ROS-induced mitochondrial dysfunction and cell death. *American Journal of Physiology. Heart and Circulatory Physiology*, *302*, H167–H179. https://doi.org/10.1152/ajpheart.00833.2011

Park, Y. Y., Lee, S., Karbowski, M., Neutzner, A., Youle, R. J., & Cho, H. (2010). Loss of MARCH5 mitochondrial E3 ubiquitin ligase induces cellular senescence through dynamin-related protein 1 and mitofusin 1. *Journal of Cell Science*, *123*, 619–626.

Parra, V., Verdejo, H. E., Iglewski, M., Del Campo, A., Troncoso, R., Jones, D., … Jana, F. (2014). Insulin stimulates mitochondrial fusion and function in cardiomyocytes via the Akt-mTOR-NFκB-Opa-1 signaling pathway. *Diabetes*, *63*, 75–88. https://doi.org/10.2337/db13-0340

Patten, D. A., Wong, J., Khacho, M., Soubannier, V., Mailloux, R. J., Pilon-Larose, K., … Slack, R. S. (2014). OPA1-dependent cristae modulation is essential for cellular adaptation to metabolic demand. *The EMBO Journal*, *33*, 2676–2691. https://doi.org/10.15252/embj.201488349

Peng, C., Rao, W., Zhang, L., Wang, K., Hui, H., Wang, L., … Fei, Z. (2015). Mitofusin 2 ameliorates hypoxia-induced apoptosis via mitochondrial function and signaling pathways. *The International Journal of Biochemistry & Cell Biology*, *69*, 29–40. https://doi.org/10.1016/j.biocel.2015.09.011

Pennanen, C., Parra, V., Lopez-Crisosto, C., Morales, P. E., del Campo, A., Gutierrez, T., … Rothermel, B. A. (2014). Mitochondrial fission is required for cardiomyocyte hypertrophy mediated by a Ca^{2+}–calcineurin signaling pathway. *Journal of Cell Science*, *127*, 2659–2671. https://doi.org/10.1242/jcs.139394

Piquereau, J., Caffin, F., Novotova, M., Prola, A., Garnier, A., Mateo, P., … Joubert, F. (2012). Down-regulation of OPA1 alters mouse mitochondrial morphology, PTP function, and cardiac adaptation to pressure overload. *Cardiovascular Research*, *94*, 408–417. https://doi.org/10.1093/cvr/cvs117

Prudent, J., Zunino, R., Sugiura, A., Mattie, S., Shore, G. C., & McBride, H. M. (2015). MAPL SUMOylation of Drp1 stabilizes an ER/-mitochondrial platform required for cell death. *Molecular Cell*, *59*, 941–955.

Rogers, M. A., Maldonado, N., Hutcheson, J. D., Goettsch, C., Goto, S., Yamada, I., … Aikawa, E. (2017). Dynamin-related protein 1 inhibition attenuates cardiovascular calcification in the presence of oxidative stress. *Circulation Research*, *121*, 220–233. https://doi.org/10.1161/CIRCRESAHA.116.310293

Rossman, M. J., Santos-Parker, J. R., Steward, C. A. C., Bispham, N. Z., Cuevas, L. M., Rosenberg, H. L., … Seals, D. R. (2018). Chronic supplementation with a mitochondrial antioxidant (MitoQ) improves vascular function in healthy older adults. *Hypertension*, *71*, 1056–1063. https://doi.org/10.1161/HYPERTENSIONAHA.117.10787

Russell, L. K., Mansfield, C. M., Lehman, J. J., Kovacs, A., Courtois, M., Saffitz, J. E., … Kelly, D. P. (2004). Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner. *Circulation Research*, *94*, 525–533. https://doi.org/10.1161/01.RES.0000117088.36577.EB

Sabbah, H. N., Gupta, R. C., Singh-Gupta, V., Zhang, K., & Lanfear, D. E. (2018). Abnormalities of mitochondrial dynamics in the failing heart: Normalization following long-term therapy with elamipretide. *Cardiovascular Drugs and Therapy*, *32*, 319–328.

Saito, T., Nah, J., Oka, S. I., Mukai, R., Monden, Y., Maejima, Y., … Sadoshima, J. (2019). An alternative mitophagy pathway mediated by Rab9 protects the heart against ischemia. *The Journal of Clinical Investigation*, *129*, 802–819. https://doi.org/10.1172/JCI122035

Saito, T., & Sadoshima, J. (2015). Molecular mechanisms of mitochondrial autophagy/mitophagy in the heart. *Circulation Research*, *116*, 1477–1490.

Salabei, J. K., & Hill, B. G. (2013). Mitochondrial fission induced by platelet-derived growth factor regulates vascular smooth muscle cell bioenergetics and cell proliferation. *Redox Biology*, *1*, 542–551.

Samant, S. A., Zhang, H. J., Hong, Z., Pillai, V. B., Sundaresan, N. R., Wolfgeher, D., … Gupta, M. P. (2014). SIRT3 deacetylates and activates OPA1 to regulate mitochondrial dynamics during stress. *Molecular and Cellular Biology*, *34*, 807–819. https://doi.org/10.1128/MCB.01483-13

Santel, A., Frank, S., Gaume, B., Herrler, M., Youle, R. J., & Fuller, M. T. (2003). Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *Journal of Cell Science*, *116*, 2763–2774.

Schiattarella, G. G., & Hill, J. A. (2015). Inhibition of hypertrophy is a good therapeutic strategy in ventricular pressure overload. *Circulation*, *131*, 1435–1447.

Schiattarella, G. G., Hill, T. M., & Hill, J. A. (2017). Is load-induced ventricular hypertrophy ever compensatory? *Circulation*, *136*, 1273–1275.

Sciarretta, S., Maejima, Y., Zablocki, D., & Sadoshima, J. (2018). The role of autophagy in the heart. *Annual Review of Physiology*, *80*, 1–26.

Sharp, W. W., Fang, Y. H., Han, M., Zhang, H. J., Hong, Z., Banathy, A., … Archer, S. L. (2014). Dynamin-related protein 1 (Drp1)-mediated diastolic dysfunction in myocardial ischemia-reperfusion injury: Therapeutic benefits of Drp1 inhibition to reduce mitochondrial fission. *The FASEB Journal*, *28*, 316–326. https://doi.org/10.1096/fj.12-226225

Shen, T., Zheng, M., Cao, C., Chen, C., Tang, J., Zhang, W., … Xiao, R. P. (2007). Mitofusin-2 is a major determinant of oxidative stress-mediated heart muscle cell apoptosis. *The Journal of Biological Chemistry*, *282*, 23,354–23,361. https://doi.org/10.1074/jbc.M702657200

Shenouda, S. M., Widlansky, M. E., Chen, K., Xu, G., Holbrook, M., Tabit, C. E., … Vita, J. A. (2011). Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus. *Circulation*, *124*, 444–453. https://doi.org/10.1161/CIRCULATIONAHA.110.014506

Sheridan, C., Delivani, P., Cullen, S. P., & Martin, S. J. (2008). Bax- or Bak-induced mitochondrial fission can be uncoupled from cytochrome C release. *Molecular Cell*, *31*, 570–585.

Shirakabe, A., Ikeda, Y., Sciarretta, S., Zablocki, D. K., & Sadoshima, J. (2016). Aging and autophagy in the heart. *Circulation Research*, *118*, 1563–1576.

Shirakabe, A., Zhai, P., Ikeda, Y., Saito, T., Maejima, Y., Hsu, C. P., … Sadoshima, J. (2016). Drp1-dependent mitochondrial autophagy plays a protective role against pressure overload-induced mitochondrial dysfunction and heart failure. *Circulation*, *133*, 1249–1263. https://doi.org/10.1161/CIRCULATIONAHA.115.020502

Sihag, S., Cresci, S., Li, A. Y., Sucharov, C. C., & Lehman, J. J. (2009). PGC-1α and ERRα target gene downregulation is a signature of the failing human heart. *Journal of Molecular and Cellular Cardiology*, *46*, 201–212.

Song, M., Chen, Y., Gong, G., Murphy, E., Rabinovitch, P. S., & Dorn, G. W. 2nd (2014). Super-suppression of mitochondrial reactive oxygen species signaling impairs compensatory autophagy in primary mitophagic cardiomyopathy. *Circulation Research*, *115*, 348–353.

Song, M., Franco, A., Fleischer, J. A., Zhang, L., & Dorn, G. W. 2nd (2017). Abrogating mitochondrial dynamics in mouse hearts accelerates mitochondrial senescence. *Cell Metabolism*, *26*, 872–883 e875.

Song, M., Gong, G., Burelle, Y., Gustafsson, A. B., Kitsis, R. N., Matkovich, S. J., & Dorn, G. W. II (2015). Interdependence of Parkin-mediated mitophagy and mitochondrial fission in adult mouse hearts. *Circulation Research*, *117*, 346–351. https://doi.org/10.1161/CIRCRESAHA.117.306859

Song, M., Mihara, K., Chen, Y., Scorrano, L., & Dorn, G. W. 2nd (2015). Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts. *Cell Metabolism*, *21*, 273–286.

Spiegel, R., Saada, A., Flannery, P. J., Burte, F., Soiferman, D., Khayat, M., … Shaag, A. (2016). Fatal infantile mitochondrial encephalomyopathy, hypertrophic cardiomyopathy and optic atrophy associated with a homozygous OPA1 mutation. *Journal of Medical Genetics*, *53*, 127–131. https://doi.org/10.1136/jmedgenet-2015-103361

Taguchi, N., Ishihara, N., Jofuku, A., Oka, T., & Mihara, K. (2007). Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *The Journal of Biological Chemistry*, *282*, 11,521–11,529.

Tsushima, K., Bugger, H., Wende, A. R., Soto, J., Jenson, G. A., Tor, A. R., … Abel, E. D. (2018). Mitochondrial reactive oxygen species in lipotoxic hearts induce post-translational modifications of AKAP121, DRP1, and OPA1 that promote mitochondrial fission. *Circulation Research*, *122*, 58–73. https://doi.org/10.1161/CIRCRESAHA.117.311307

Twig, G., Elorza, A., Molina, A. J., Mohamed, H., Wikstrom, J. D., Walzer, G., … Las, G. (2008). Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *The EMBO Journal*, *27*, 433–446. https://doi.org/10.1038/sj.emboj.7601963

van der Bliek, A. M., Shen, Q., & Kawajiri, S. (2013). Mechanisms of mitochondrial fission and fusion. *Cold Spring Harbor Perspectives in Biology*, *5*(6), a011072.

Varanita, T., Soriano, M. E., Romanello, V., Zaglia, T., Quintana-Cabrera, R., Semenzato, M., … Scorrano, L. (2015). The OPA1-dependent

mitochondrial cristae remodeling pathway controls atrophic, apoptotic, and ischemic tissue damage. *Cell Metabolism*, *21*, 834–844. https://doi.org/10.1016/j.cmet.2015.05.007

Vasquez-Trincado, C., Garcia-Carvajal, I., Pennanen, C., Parra, V., Hill, J. A., Rothermel, B. A., & Lavandero, S. (2016). Mitochondrial dynamics, mitophagy and cardiovascular disease. *The Journal of Physiology*, *594*, 509–525. https://doi.org/10.1113/JP271301

Wai, T., Garcia-Prieto, J., Baker, M. J., Merkwirth, C., Benit, P., Rustin, P., … Langer, T. (2015). Imbalanced OPA1 processing and mitochondrial fragmentation cause heart failure in mice. *Science*, *350*, aad0116. https://doi.org/10.1126/science.aad0116

Wang, H., Song, P., Du, L., Tian, W., Yue, W., Liu, M., … Zhou, J. (2011). Parkin ubiquitinates Drp1 for proteasome-dependent degradation: Implication of dysregulated mitochondrial dynamics in Parkinson disease. *The Journal of Biological Chemistry*, *286*, 11,649–11,658. https://doi.org/10.1074/jbc.M110.144238

Wang, J., Chen, H., Liu, Y., Zhou, W., Sun, R., & Xia, M. (2015). Retinol binding protein 4 induces mitochondrial dysfunction and vascular oxidative damage. *Atherosclerosis*, *240*, 335–344.

Wang, J. X., Jiao, J. Q., Li, Q., Long, B., Wang, K., Liu, J. P., … Li, P. F. (2011). miR-499 regulates mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1. *Nature Medicine*, *17*, 71–78. https://doi.org/10.1038/nm.2282

Wang, K., Long, B., Jiao, J. Q., Wang, J. X., Liu, J. P., Li, Q., & Li, P. F. (2012). miR-484 regulates mitochondrial network through targeting Fis1. *Nature Communications*, *3*, 781. https://doi.org/10.1038/ncomms1770

Wang, Q., Zhang, M., Torres, G., Wu, S., Ouyang, C., Xie, Z., & Zou, M. H. (2017). Metformin suppresses diabetes-accelerated atherosclerosis via the inhibition of Drp1-mediated mitochondrial fission. *Diabetes*, *66*, 193–205. https://doi.org/10.2337/db16-0915

Watanabe, T., Saotome, M., Nobuhara, M., Sakamoto, A., Urushida, T., Katoh, H., … Hayashi, H. (2014). Roles of mitochondrial fragmentation and reactive oxygen species in mitochondrial dysfunction and myocardial insulin resistance. *Experimental Cell Research*, *323*, 314–325. https://doi.org/10.1016/j.yexcr.2014.02.027

Waterham, H. R., Koster, J., van Roermund, C. W., Mooyer, P. A., Wanders, R. J., & Leonard, J. V. (2007). A lethal defect of mitochondrial and peroxisomal fission. *The New England Journal of Medicine*, *356*, 1736–1741.

Yu, T., Robotham, J. L., & Yoon, Y. (2006). Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 2653–2658.

Yu, T., Sheu, S. S., Robotham, J. L., & Yoon, Y. (2008). Mitochondrial fission mediates high glucose-induced cell death through elevated production of reactive oxygen species. *Cardiovascular Research*, *79*, 341–351.

Zepeda, R., Kuzmicic, J., Parra, V., Troncoso, R., Pennanen, C., Riquelme, J. A., … Lavandero, S. (2014). Drp1 loss-of-function reduces cardiomyocyte oxygen dependence protecting the heart from ischemia-reperfusion injury. *Journal of Cardiovascular Pharmacology*, *63*, 477–487. https://doi.org/10.1097/FJC.0000000000000071

Zhang, H., Wang, P., Bisetto, S., Yoon, Y., Chen, Q., Sheu, S. S., & Wang, W. (2017). A novel fission-independent role of dynamin-related protein 1 in cardiac mitochondrial respiration. *Cardiovascular Research*, *113*, 160–170. https://doi.org/10.1093/cvr/cvw212

Zhao, L., Zou, X., Feng, Z., Luo, C., Liu, J., Li, H., … Liu, J. (2014). Evidence for association of mitochondrial metabolism alteration with lipid accumulation in aging rats. *Experimental Gerontology*, *56*, 3–12. https://doi.org/10.1016/j.exger.2014.02.001

Zhao, T., Huang, X., Han, L., Wang, X., Cheng, H., Zhao, Y., … Zheng, M. (2012). Central role of mitofusin 2 in autophagosome-lysosome fusion in cardiomyocytes. *The Journal of Biological Chemistry*, *287*, 23,615–23,625. https://doi.org/10.1074/jbc.M112.379164

Zhao, Y. X., Cui, M., Chen, S. F., Dong, Q., & Liu, X. Y. (2014). Amelioration of ischemic mitochondrial injury and Bax-dependent outer membrane permeabilization by Mdivi-1. *CNS Neuroscience & Therapeutics*, *20*, 528–538.

Zorzano, A., Liesa, M., & Palacin, M. (2009). Role of mitochondrial dynamics proteins in the pathophysiology of obesity and type 2 diabetes. *The International Journal of Biochemistry & Cell Biology*, *41*, 1846–1854. https://doi.org/10.1016/j.biocel.2009.02.004

Zuchner, S., Mersiyanova, I. V., Muglia, M., Bissar-Tadmouri, N., Rochelle, J., Dadali, E. L., … Vance, J. M. (2004). Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nature Genetics*, *36*, 449–451. https://doi.org/10.1038/ng1341

How to cite this article: Forte M, Schirone L, Ameri P, et al. The role of mitochondrial dynamics in cardiovascular diseases. *Br J Pharmacol*. 2021;178:2060–2076. https://doi.org/10.1111/bph.15068