REVIEW

Post-Translational Modification of Drp1 is a Promising Target for Treating Cardiovascular Diseases

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Abstract

Mitochondria are essential for cell growth, fission, differentiation, and survival, particularly in undivided cells with high energy requirements, such as cardiomyocytes. The morphology and position of mitochondria change with the activity of mitochondrial fission proteins and mitochondrial fusion proteins. These regulatory mechanisms substantially affect cardiomyocyte energy supply and normal function. In mitochondrial fission, dynamin-related protein 1 (Drp1) is involved in the separation and degradation of damaged mitochondria, and accurately regulates mitochondrial renewal and number. Recent studies have revealed a variety of post-translational modification (PTMs) of Drp1, including phosphorylation, SUMOylation, acetylation, O-GlcNAcylation, and S-sulfhydration. These modifications ensure that Drp1 continues to function normally in various signaling pathways, by modulating its activity, stability, and subcellular localization. This article provides an overview of the relationship between Drp1 PTMs and cardiovascular diseases such as heart failure, myocardial infarction, and myocardial ischemia-reperfusion, and describes how these modifications can be targeted and regulated, to help guide cardiovascular disease treatment.

Keywords: post-translational modifications; cardiovascular diseases; DRP1; Mitochondrial fission

Introduction

Mitochondria, which provide energy for cellular biological processes, are abundant in muscle tissue, particularly in the heart, and have important effects on cardiomyocyte pathology and physiology [1]. Adult myocardial mitochondria account for approximately 30% of the total cell volume and produce large amounts of ATP through oxidative phosphorylation, thereby maintaining contractile function [2].

The morphology, number, and size of mitochondria constantly change to adapt to the body's environment and needs. To maintain the dynamic balance of mitochondria and the stability of energy provision, cardiomyocytes regulate their biological state through mitochondrial fusion and fission, as well as mitochondrial biogenesis and mitochondrial-specific mitophagy. The field of mitochondrial dynamics was developed to study these specific mechanisms [3–5]. Studies increasingly indicate that mitochondrial dynamics is closely associated with mitochondrial function. Disruption of the dynamic balance of mitochondrial fission and fusion leads to structural alterations and dysfunction; moreover,

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mitochondrial fission and fusion are early responses to mitochondrial dysfunction that maintain normal form and function [6, 7].

Mitochondrial dynamics is regulated by a variety of proteins, through changes in mitochondrial number and activity. Among these proteins, mammalian dynamin-associated protein 1 (Drp1) is a major regulator of mitochondrial fission [8]. Drp1-dependent mitochondrial fission is a complex process that regulates the complex pathophysiological processes of cardiomyocytes and responds to various cardiac diseases through numerous mechanisms, such as effects on cellular energy metabolism, regulation of intracellular calcium levels, and the production of reactive oxygen species (ROS) and proapoptotic proteins [9, 10]. For example, cardiac-specific knockdown of Drp1 leads to dilated cardiomyopathy and rapid death in mice [11]. In Drp1 knockdown cardiomyocytes, mitochondria show increased fusion, accumulation of ubiquitination proteins, decreased aerobic respiration, and inadequate cellular energy supply [12]. Moreover, Drp1 is an important regulator of cardiomyocytes in response to various stress conditions, such as hyperglycemia, hypoxia, and oxidative stress [13–16]. However, the regulatory role of Drp1 on mitochondrial fission in cardiomyocytes is reflected not only at the protein level but also at the activity level, through strict regulation by post-translational modifications (PTMs) [17, 18]. For example, during reperfusion, Drp1 Ser-637 is activated by increased dephosphorylation, which in turn leads to mitochondrial translocation, thereby resulting in increased undesirable mitochondrial fission [19]. In contrast, the calcineurin inhibitor FK506 prevents dephosphorylation of Drp1 Ser-637 and protects cardiac function during ischemia/reperfusion (I/R) [20]. Herein, we review recent studies on PTMs of Drp1 in the pathogenesis of cardiovascular diseases, such as heart failure, myocardial infarction, and myocardial fibrosis, and describe the strategies or approaches to treat these cardiovascular diseases by targeting Drp1 PTMs. The potential of Drp1 PTMs as a key target for the future treatment of cardiovascular diseases is highlighted.

Structure and Function of Drp1

Drp1 is a GTP binding protein in the Dynein family, which includes the yeast Recombinant Dynamin 1 (DNM1), and the mammalian dynamin I, II, and III. The domain structure of Drp1 includes the N-terminal GTPase domain, middle domain, variable domain (VD, also known as B-insert), and C-terminal GTPase effector domain (GED) [21]. Crystal structures have indicated that the VDs act as hinges by forming T-dimers or tetramers, and effectively binding the target membrane [22]. Drp1 functions in mitochondrial fission in four steps: first, Drp1 translocates from the cytosol to the outer mitochondrial membrane (OMM); second, recombinant Drp1 is formed; third, Drp1 GTPase is activated, thus leading to remodeling and contraction of the mitochondrial membrane; and finally, mitochondrial fission is induced, thereby producing offspring mitochondria [23, 24]. To date, four OMM receptors and/or adapters have been identified that recruit Drp1 from the cytosol to the OMM for fission: mitochondrial dynamics proteins 49 and 51 (MiD49 and MiD51), mitochondrial fission factor (MFF), and mitochondrial fission 1 protein (Fis1) (Figure 1). In contrast, the endoplasmic reticulum also plays a role in the recruitment of Drp1 by transferring calcium ions into the mitochondria [25]. Drp1 is inextricably associated with disease development. In humans, fission-defective Drp1 mutations are associated with fatal microcephaly [26]. Mouse embryonic cells with Drp1 knockout have markedly downregulated ATP levels and do not survive [27]. Similarly, in neonatal rat ventricular myocytes, deletion of Drp1 leads to the accumulation of damaged mitochondria, decreases intracellular ATP, and leads to apoptosis [28]. Beyond Drp1 protein levels, the role of Drp1 depends on the dynamic balance of its activity, in a process regulated by various mechanisms, such as ROS, CDK1, PKCδ, and calcineurin (CaN). Regulation of Drp1 activity maintains normal mitochondrial fission, thus effectively controlling relevant disease process. However, how to precisely alter Drp1 activity to achieve therapeutic effects remains to be clarified.

PTMs of Drp1 in the Heart

PTMs are covalent processing events that alter the biochemical properties of a protein by proteolytic cleavage and the addition of modifying groups to one or more amino acids via enzyme catalysis, and which play key roles in many biological processes.
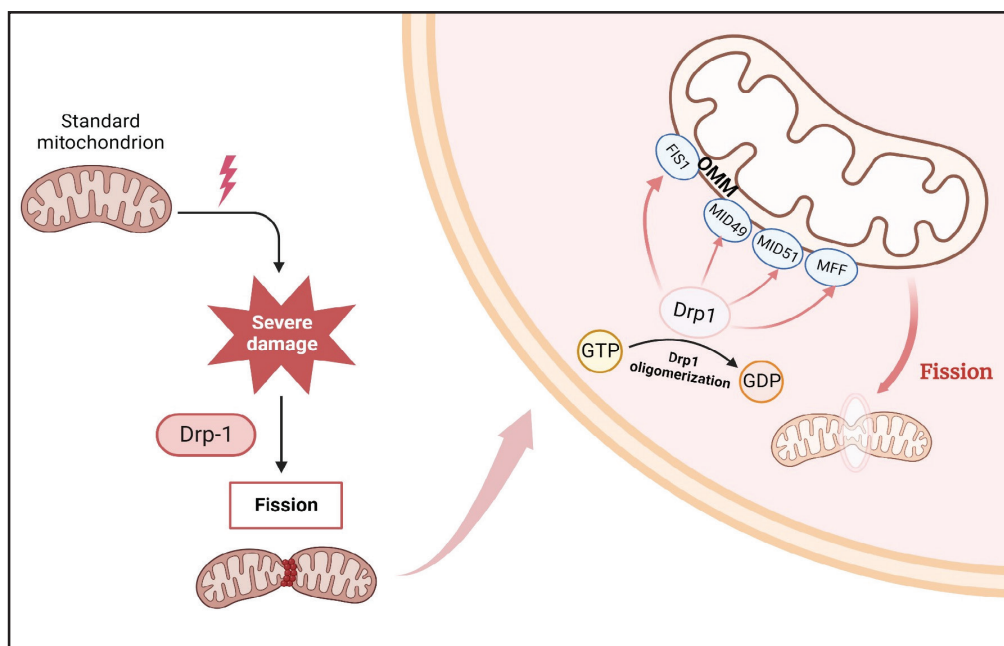
Figure 1 Cytosolic Dynamin Related Protein 1 (Drp1) Monomers are Recruited to the Outer Mitochondrial Membrane by the Adaptor Proteins Mitochondrial Fission 1 Protein (FIS1), Mitochondrial Dynamics Protein 49 (MID49), MID51, and Mitochondrial Fission Factor (MFF), thereby Facilitating the Formation of Helical Ring Drp1 Oligomers. GTP hydrolysis by Drp1 stimulates outer mitochondrial membrane constriction and subsequent scission. Drp1: Dynamin-Related Protein 1; GTP: guanosine triphosphate; FIS1: Mitochondrial Fission 1 Protein; MID49: mitochondrial dynamics proteins 49; MID51: mitochondrial dynamics proteins 51; MFF: Mitochondrial Fission Factor; OMM: Outer mitochondrial membrane (figure created with BioRender.com).

To date, more than 450 protein PTMs have been identified, including phosphorylation, ubiquitination, SUMOylation, acetylation, methylation, and O-GlcNAcylation. These PTMs alter the activity, stability, protein interactions, and intracellular localization of target proteins [29]. Most PTMs are reversible and quantitatively alterable, and multiple PTMs can also regulate one another. This regulation can be competitive or facilitative, as in the case of the Recombinant Mothers Against Decapentaplegic Homolog 4 (SMAD4) protein, wherein phosphorylation and O-GlcNAcylation compete with each other for modification sites [30]. In another example, ubiquitination and acetylation of the cancer suppressor gene P53 can be mutually blocked at certain specific sites, whereas phosphorylation can promote or inhibit acetylation, which is site-specific [31]. Furthermore, the same modification at different sites can have different effects on protein function. The reversibility of PTMs is the key to the rapid adaptation of cells to various environmental changes [32].

In normal cells, Drp1 is modified by various PTMs that influence specific functions of Drp1, thereby maintaining cellular environmental homeostasis in a given environment. In addition, crosstalk among different PTMs on Drp1 can be regulated, thus allowing cells to adapt to various changes in different environments [33]. Abnormalities in PTMs can lead to abnormal activity of Drp1, thereby affecting cellular processes such as mitochondrial fission and energy supply, and consequently inducing or exacerbating heart disease [34]. PTMs provide a new perspective on the regulation of Drp1 protein levels/activity, given the presence of numerous PTMs targets (Figure 2). Herein, we review the functions of PTMs such as SUMOylation, phosphorylation, acetylation, O-GlcNAcylation, and S-sulfhydration in regulating Drp1; describe their correlations with cardiac diseases; and highlight that PTMs targeting Drp1 may have great potential in future heart disease-related therapies.

Phosphorylation

Phosphorylation is the most prominent and extensively studied PTM of Drp1. Depending on the
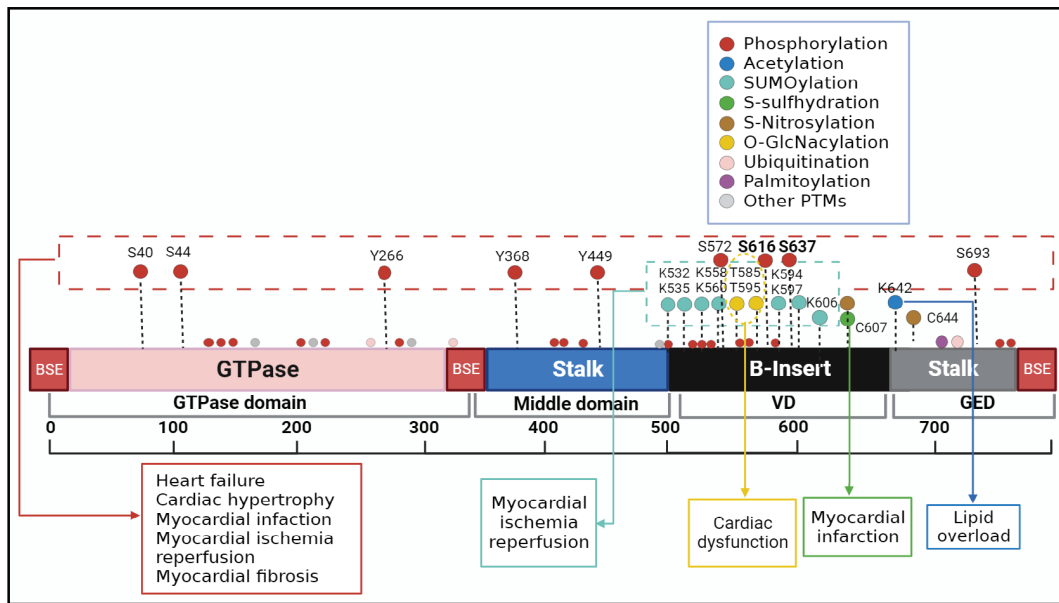
Figure 2 Chart Showing Drp1 Structure, PTM Distribution, and Related Cardiac Diseases.
The domain structure of Drp1 includes the N-terminal GTPase domain, middle domain, B-insert, and C-terminal GTPase effector domain. Amino acid residues without site information are indicated only from proteomics and mass spectrometry data. Phosphorylation sites include Ser-40, Ser-44, Y266, Y368, Y449, Ser-572, Ser-616, Ser-637, and Ser-693, affecting the related cardiac diseases of heart failure, cardiac hypertrophy, myocardial infarction, myocardial ischemia reperfusion, and myocardial fibrosis. Acetylation sites include K642, affecting the related heart disease of lipid overload. SUMOylation sites include K532, K535, K558, K560, K594, K597, and K606, affecting the related heart disease of myocardial ischemia reperfusion. S-sulfhydration sites include C607 and C644, affecting the related cardiac disease of cardiac infarction. O-GlcNacylation sites include T585 and T595, affecting the related cardiac disease of cardiac dysfunction. S-nitrosylation sites include C607. Different colored dots represent different post-translational modifications, as indicated in the box in the upper right corner. The text connected by the dotted boxes indicates the diseases associated with these loci, which are explained in the figure notes. BSE, bundle signaling element; GED, GTPase effector domain; VD, variable domain (figure created with BioRender.com).

modification sites, Drp1 phosphorylation can exert either activating or inhibiting effects, and many phosphorylation sites have been identified, such as Ser-579, Ser-40, Ser-585, Ser-44, Ser-592, Ser-656, Ser-616, Ser-637, and Ser-693 [35]. Among them, Ser-616 and Ser-637 (corresponding to mouse Ser-579 and Ser-600, respectively) are the two most studied phosphorylation sites involved in the regulation of mitochondrial fission [36]. The role of Drp1 phosphorylation in different cardiac disorders is discussed in the following section.

Numerous studies have shown that mitochondrial function has important effects on the development of heart failure. Drp1 phosphorylation is indispensable for the myocardial energy supply. For example, fibronectin increases the phosphorylation level of the Drp1 Ser-616 site via FAK-ERK1/2-Drp1, thus increasing the oxygen consumption rate and ATP content of neonatal rat ventricular myocytes. Inhibition of the FAK-ERK1/2-Drp1 pathway results in a shortage of cellular energy, thereby decreasing cardiac function. Adrenaline has been experimentally shown to activate this pathway [37]. In heart failure with preserved ejection fraction (HFpEF), myocardial-specific overexpression of PTEN-induced kinase 1 (PINK1) activates phosphorylation of Drp1 Ser-616 and promotes mitochondrial fission, thereby slowing the progression of hypertension-induced HFpEF [38]. However, Drp1 hyperphosphorylation has also been demonstrated to be a detrimental factor in heart failure. For instance, insulin-like growth factor II receptor (IGF-IIR) mediates extracellular regulated protein kinase (ERK) activation, thus resulting in hyperphosphorylation of the Drp1 Ser-616 site and Drp1 translocation to mitochondria. Accordingly, mitochondrial fission and dysfunction enhance the ability of Rab9-dependent autophagosomes to recognize and engulf damaged mitochondria, thus ultimately decreasing cardiomyocyte viability [39]. Blocking IGF-IIR signaling effectively inhibits cardiomyocyte hypertrophy and apoptosis [37], and inhibiting Drp1

activity by regulating Drp1 phosphorylation levels attenuates the loss of mitochondrial transmembrane potential and the decrease in cell survival caused by IGF-IIR activation [39]. YiQiFuMai powder injection significantly decreases coronary artery ligation-induced heart failure by improving mitochondrial morphology, increasing mitochondrial membrane potential, decreasing Drp1 phosphorylation, and protecting mitochondrial function [40]. The above experiments have indicated that the phosphorylation of Drp1 is closely associated with the development of heart failure, and the regulation of Drp1 phosphorylation levels under specific conditions may be important in protecting cardiac function.

Hypertension or acute coarctation of the aorta that results in pressure overload in the left ventricle can cause pathological myocardial hypertrophy. Through compensation, abnormal metabolic, structural, and functional signals are generated, which in turn result in abnormal cardiac function [41]. Therefore, inhibiting myocardial hypertrophy is a major topic of current research. Drp1 Ser-622 is phosphorylated by PKC-δ in rat cardiomyocytes, thus leading to myocardial hypertrophy [42]. Leptin-induced cardiomyocyte hypertrophy is associated with calcium-regulated neurophosphatase-mediated Drp1 Ser-637 phosphorylation, and inhibition of Drp1 phosphorylation attenuates the associated myocardial hypertrophy [43]. By activating calcium-regulated phosphatases, norepinephrine promotes myocardial hypertrophy associated with phosphorylation of Drp1 Ser-637 [44]. Low doses of bisphenol A cause myocardial hypertrophy through calcium homeostasis via the calcium-regulated neurophosphatase-Drp1 Ser-637 signaling pathway [45]. Although these studies appear to indicate that elevated Drp1 phosphorylation promotes myocardial hypertrophy, recombinant signal transducer and activator of transcription 1 (STAT1) has been shown to enhance mitochondrial function and cardiomyocyte function through the Uncoupling protein 2 (Ucp2)/P-Drp1 signaling pathway, thus inhibiting myocardial hypertrophy [46]. According to the above studies, Drp1 phosphorylation promotes myocardial hypertrophy in myocardial structure, whereas Drp1 phosphorylation inhibits myocardial hypertrophy in the energy supply and consequently inhibits myocardial hypertrophy. Because of the specific roles played by Drp1

phosphorylation in different pathways, complex functional regulatory crosstalk may exist, and regulation of Drp1 phosphorylation levels may be a valuable target for cardiac disease treatment.

Massive cardiomyocyte death results from acute ischemic episodes that cause myocardial infarction [47]. Drp1 is activated in the infarct zone of mouse heart tissue, thus resulting in substantial mitochondrial fragmentation and cardiac dysfunction [48]. Inhibiting Drp1 activity can decrease mitochondrial metabolic disturbances and fragmentation, thereby protecting the heart against the adverse effects of myocardial infarction [49, 50]. Hypothermia promotes myocardial mitochondrial lengthening by inhibiting Drp1 Ser-616 phosphorylation, and consequently decreasing oxygen consumption and ischemic injury [51]. Secreted frizzled-related protein 5 (Sfrp5), a protective regulatory protein in coronary heart disease, decreases Drp1 Ser-616 phosphorylation levels and significantly diminishes infarct size [52]. Similarly to Sfrp5 action, uncoupling protein 2 (UCP2) overexpression during myocardial infarction inhibits Drp1 Ser-616 phosphorylation, thereby decreasing apoptosis and improving cardiac function [53]. By disrupting mitochondrial fission and activating AMPK, sirtuin 3 (SIRT3) delays myocardial injury after myocardial infarction by promoting Drp1 dephosphorylation and Drp1 phosphorylation [54]. On the basis of the above studies, regulating Drp1 phosphorylation levels substantially enhance cardiomyocyte survival after myocardial infarction.

After myocardial infarction, emergency opening of occluded coronary arteries restores blood flow to the ischemic tissue. Nonetheless, myocardial tissue is destroyed within the first few minutes of reperfusion, thus resulting in sustained cardiac injury. This occurrence, referred to as I/R injury, is responsible for the expansion of the infarcted region [55]. A growing body of evidence indicates that excessive mitochondrial fission contributes to cardiomyocyte death after I/R [56]. During ischemia and reperfusion, mitochondrial fission is activated, thus resulting in high mitochondrial production of ROS, calcium overload, and over-opening of mitochondrial permeability transition pores [36]. In mouse models of myocardial I/R injury, elevated levels of phosphoglycerate mutase 5 (PGAM5) protein cause myocardial dephosphorylation of Drp1 Ser-637 and increased phosphorylation of Drp1 Ser-616, which

may lead to mitochondrial fragmentation and dysfunction. This response is an early manifestation of myocardial I/R injury symptoms, and PGAM5 knockdown inhibits Drp1 Ser-637 dephosphorylation in mice. Although PGAM5 deletion does not affect Drp1 Ser-616 phosphorylation, it nonetheless partially inhibits mitochondrial fragmentation and decreases I/R injury [57, 58]. In mouse models of I/R injury, the AMP-activated Protein Kinase (AMPK) agonist AICAR ameliorates isolated cardiac function, and decreases arrhythmia incidence and myocardial infarct size, by increasing AMPK activity, thereby decreasing Drp1 phosphorylation at Ser-616 and increasing Drp1 phosphorylation at Ser-637 [59]. On the basis of the results of these studies, mitochondria and cardiomyocytes under I/R injury might be protected through modulation of Drp1 phosphorylation.

In wound healing after myocardial injury, mitochondrial fission plays a crucial role in stimulating fibroblast proliferation and collagen synthesis. Nevertheless, excessive matrix deposition results in maladaptive fibrous remodeling and disruption of electrical signals, thus ultimately leading to cardiac dysfunction [60]. Transforming growth factor-β (TGF-β) stimulates fibroblast proliferation, migration, and extracellular matrix synthesis, thereby leading to myocardial fibrosis. Inhibition of Drp1 suppresses these TGF-β-stimulated processes [61], thereby providing an opportunity to modulate myocardial fibrosis through Drp1. One study has shown that 3-sn-lysophosphatidylcholine-induced protein kinase C (PKC) interacts with Drp1 and phosphorylates Drp1 Ser-616, thereby enhancing mitochondrial fission and depolarization mediated by Drp1. Inhibiting this pathway decreases fibroblast activation and collagen accumulation in the heart, thus diminishing fibrosis [62]. Consequently, inhibiting Drp1 phosphorylation may decrease excessive activation of fibroblast mitochondrial fission under cardiac stress, heart remodeling, and fibrosis.

SUMOylation

SUMO is a small ubiquitin-like modifier attached to substrate proteins through an enzyme linked reaction. SUMOylation entails a reversible and dynamic modification of lysine residues in substrate proteins, e.g., Lys-532, Lys -535, Lys-594, Lys-608, Lys-606,

Lys-558, Lys-568, and Lys-597) [63], thereby often altering proteins' subcellular localization or protecting them against ubiquitin-triggered damage. In the past decade, many studies have shown that SUMOylation contributes to cardiac perfusion [64, 65]. Furthermore, SUMOylation is involved in the regulation of Drp1 activity [66]. According to current understanding, SUMO2/3 SUMOylates Drp1, and SUMO-specific protease 3 (SENP3) and SUMO-specific protease 5 (SENP5) exert desumoylation, whereas SUMO1-mediated Drp1 SUMOylation is removed by SUMO-specific protease 2 (SENP2) [67, 68]. SENP3-mediated Drp1 desumoylation facilitates Drp1's interaction with MFF outside the mitochondrial membrane, and the interaction of Drp1 mutation at this SUMOylation site with MFF increases mitochondrial binding [67]. However, unlike the facilitative effect of SNEP3 on mitochondrial fission, desumoylation of Drp1 in mice overexpressing SENP5 results in mitochondrial dysfunction and myocardiopathy, thus confirming the deleterious effect of Drp1 desumoylation on heart failure [69]. Additionally, SUMOylated Drp1 protects against myocardial I/R injury caused by zinc. Zinc-mediated Drp1 SUMOylation enhances mitophagy during reperfusion, thereby decreasing ROS and myocardial damage [70]. Findings have indicated that Drp1 SUMOylation exerts a considerable influence on cardiac function; however, how it might meet clinical therapeutic standards remains to be determined.

S-nitrosylation

Cysteine plays a crucial role in redox signal conduction, which converts oxidant signals into biological responses. In reversible cysteine PTMs, the messenger nitric oxide (NO) is covalently coupled to cysteine residues of target proteins, thus resulting in the formation of protein S-nitrosylation, a redox switch involved in several pathological conditions, such as I/R, synaptic transmission, cancer, and muscle dysfunction [71]. In neuronal cells, C644 S-nitrosylation in the Drp1 GED structural domain has been shown to increase the formation of Drp1 oligomers, enhance GTPase activity, and alter protein conformation, thereby inducing mitochondrial disruption, synaptic damage, and bioenergetic failure, and exacerbating neurodegenerative diseases, such as Alzheimer's and Huntington's diseases

[35]. However, S-nitrosylation of Drp1 has no direct effect on Drp1 activity, instead, Drp1 C644 S-nitrosylation increases mitochondrial fission by promoting Drp1 Ser-616 phosphorylation [72]. Findings have indicated that aberrant NO production leads to mitochondrial and synaptic dysfunction. In the cardiovascular system, several proteins that undergo S-nitrosylation, including calcium homeostasis-related proteins, mitochondrial proteins, hemoglobin, myosin, and ion channels that regulate contractility, are targets of endogenous and exogenous NO S-nitrosylation [73]. Nevertheless, controversy remains regarding the function of Drp1 S-nitrosylation in relation to cardiac disease, and further research is required to confirm the cardiac relevance of Drp1 S-nitrosylation.

S-sulfhydration

Hydrogen sulfide (H_2S) is a product of cardiac cystathionine γ-lyase (CSE) metabolism and is a known biological active gas substance [74]. The physiological roles of H_2S include modulation of vasodilation, anti-inflammatory, antioxidant, and angiogenesis functions [75]. During cardiac injury, endogenous production of H_2S decreases, and insufficient production results in heart disease [76]. H_2S is believed to have anti-atherogenic properties that prevent atherosclerosis [77]. Studies have shown that cardiac-specific CSE gene overexpression mice maintains cardiac structure and function after transverse aortic constriction [78]. Additionally, exogenous sulfide treatment to increase H_2S levels has also demonstrated effective myocardial protection in mouse models of Myocardial ischemia-reperfusion injury [79]. Several studies have demonstrated that S-sulfhydration of H_2S directly competes with S-nitrosylation of NO for binding cysteine 607 of Drp1. Drp1 S-sulfhydration regulates Drp1 phosphorylation and GTPase activity, and inhibits its activity and translocation to mitochondria, thus attenuating mitochondrial overfission. In addition, Drp1 S-sulfhydration attenuates cardiomyocyte apoptosis by decreasing the interaction between Drp1 and Voltage Dependent Anion Channel Protein 1 (VDAC1). The non-S-sulfhydration of Drp1 (mutation of cysteine 607 to alanine) inhibits the protective effect of H_2S on cardiac function [80].

S-sulfhydration is a novel Drp1 PTM with potential therapeutic value for cardiovascular diseases.

Acetylation

Acetylation is another major PTM in cells [81]. During the late 1990s, the first histone acetyltransferases and histone deacetylases were cloned [82, 83]. According to recent research, heart failure is associated with hyperacetylation of myocardial mitochondrial proteins [84, 85], possibly because of an increase in acetyl coenzyme A utilization or disruption of NAD+ homeostasis, and subsequent inhibition of SIRT3 deacetylase activity [86]. A critical factor in heart failure is that protein hyperacetylation may lead to metabolic remodeling [87]. Protein acetylation rates vary according to nutritional status. Nutritional overload has been shown to cause Drp1 to become acetylated at lysine 642 (K642), thus leading to cardiomyocyte dysfunction and death. Excess lipid supply creates an intracellular environment that promotes Drp1 acetylation and consequently leads to increased activity of Drp1 and mitochondrial translocation [88]. Therefore, Drp1 acetylation can cause cardiomyocyte death and dysfunction as a result of metabolic stress. Drp1 acetylation may be a key PTM contributing to lipid overload-induced cardiac dysfunction and may be a potential therapeutic target.

O-GlcNAcylation

O-linked N-acetyl-β-D-glucosamine (O-GlcNAc) is a PTM commonly found on the serine and/or threonine residues of nuclear and cytoplasmic proteins. O-GlcNAcylation and removal of GlcNAc from O-proteins are catalyzed by O-GlcNAc transferase (OGT) and O-GlcNAc hydrolase (OGA), respectively [89, 90]. Abnormal O-GlcNAcylation has been implicated in the pathogenesis of a variety of diseases, including cancer, diabetes, neurodegenerative and cardiovascular diseases [91–93]. Cardiac-specific OGT knockout mice show increased cardiac dysfunction after myocardial infarction, thus suggesting that O-GlcNAcylation has a crucial role in preserving cardiac function [94]. Drp1 is acylated by O-GlcNAc, and increasing O-GlcNAcylation augments the level of the GTP-bound active form of Drp1, and induces

translocation of Drp1 from the cytoplasm to mito-chondria [95]. High glucose treatment in cardiomy-ocytes induces O-GlcNAcylation at Drp1 threonine 585 (T585) and threonine 586 (T586), thus decreasing Drp1 phosphorylation at Ser-637. Consequently, Drp1 activity and mitochondrial breakage further increase, thereby inhibiting OGA and impairing mitochondrial function in cardiomyocytes [95]. Alternatively, knock-down of OGT decreases Drp1 Ser-637 phosphoryla-tion levels and increases Drp1 translocation from the cytoplasm to the mitochondria in mouse models of cerebral I/R injury. After OGT knockdown, infarct volume and neurological function scores significantly increase, as do levels of cleaved caspase-3 and neu-ronal apoptosis [96]. Accordingly, O-GlcNAcylation of Drp1 plays different roles under various pathologi-cal conditions and may contribute to diabetes-induced mitochondrial dysfunction, such as diabetic cardio-myopathy. However, how O-GlcNAcylation of Drp1 functions during myocardial ischemia remains to be determined.

Ubiquitination

Ubiquitination is a key PTM that regulates biological functions through the covalent attachment of ubiqui-tin, consisting of 76 amino acids, to target proteins, thereby altering their structure or tagging them for proteasome degradation [97]. During ubiquitina-tion, three types of enzymes are involved: ubiqui-tin activating enzymes (E1S), ubiquitin conjugating enzymes (E2S), and ubiquitin-protein ligases (E3S). Ubiquitin is first activated by E1S and adenosine triphosphate. Activated ubiquitin is then transferred to E2S and is subsequently covalently attached to the target residues on the substrate via E3S [98]. Drp1 has been shown to be ubiquitinated by E3S, including the OMM-anchored E3 ubiquitin-protein ligase (MARCH5) and Parkin [99, 100]. As a result of ubiquitination of Drp1, March5 participates in the regulation of mitochondrial morphology, thus leading to Drp1 degradation by proteasomes and decreased mitochondrial fission [101]. However, the ubiquitination of MARCH5-Drp1 can also promote mitochondrial fission by facilitating the recruit-ment of Drp1 to specific fission sites [102]. Recent studies have demonstrated that Drp1 regulates MARCH5 [103], thus completing the mechanism

through which Drp1 is ubiquitinated. Parkin-Drp1 ubiquitination further promotes Drp1 degradation and decreases mitochondrial fission activity through a proteasome-dependent pathway [100]. This find-ing is consistent with observations that a decrease in Drp1 degradation caused by Parkin knockout or pathogenic mutations results in excessive mitochon-drial fission and ultimately disease [35]. Currently, no evidence indicates that Drp1 ubiquitination con-tributes to cardiac diseases. Whether Drp1 ubiqui-tination is associated with altered cardiac structure and function remains to be determined, and research in this area has great potential.

S-palmitoylation

In S-palmitoylation, palmitate is attached to cysteine residues of proteins via a reversible thi-oester bond by palmitoyl transferase of a zinc fin-ger DHHC domain-containing protein (ZDHHC). This process regulates protein activity, stability, transport, and protein-protein interactions [104]. Recent studies have shown that S-palmitoylation is involved in mitochondrial fission-fusion regulation through a related signaling pathway [105]. Drp1 S-palmitoylation is a key mechanism in Drp1 trans-location to mitochondria and the normal fission-fusion process. In addition to altering Drp1 activ-ity, S-palmitoylation affects mitochondrial ATP production and switches the glycolytic glutamate and γ-aminobutyric acid cycles [106]. A direct pro-tein-protein interaction has been observed between Zdhhc13 and Drp1 in vivo and in vitro. In mice car-rying the spontaneous stealth mutant Zdhhc13 gene (LUC), Drp1 S-palmitoylation is decreased, thus resulting in abnormal co-localization of Drp1 with mitochondria, and altered mitochondrial morphol-ogy and distribution. Ultimately, the mitochondrial dynamics is disrupted, thus leading to abnormal mitochondrial mitosis, and resulting in cancer or cardiac arrhythmias [106]. Although only several studies have examined Drp1 S-palmitoylation, pre-liminary evidence suggests that plays a key role in cardiovascular disease. The altered status of Drp1 S-palmitoylation in different diseases and the under-lying regulatory mechanisms remain to be studied, particularly in the cardiac field, wherein altered Drp1 activity has been associated with a series of

important pathophysiological processes. Thus, S-palmitoylation of Drp1 has high research value.

Targeting Drp1-Modified Pathways for Treatment of Heart Diseases

Cardiac dysfunction can feasibly be prevented and treated by targeting Drp1 modifications with drugs, because Drp1-dependent mitochondrial division significantly contributes to cardiac disease development and progression [107]. Drp1 PTMs have been shown to be altered by several drugs. Mitochondrial division inhibitor 1 (mdivi-1) has been identified as a small molecule inhibitor of Drp1. In diabetic cardiomyopathy, mdivi-1 treatment significantly decreases angiotensin II-induced Drp1 Ser-616 phosphorylation and subsequently myocardial I/R injury [19, 108], and increases ATP levels and mitochondrial complex (I, IV, and V) activity levels. However, mdivi-1 may promote the accumulation of damaged mitochondria and impair cardiac function after long-term use in the treatment of myocardial hypertrophy and diabetic cardiomyopathy [109]. In addition, an astragaloside IV derivative (LS-102) has been found to decrease phosphorylation of Drp1Ser-616 and increase phosphorylation of Drp1Ser-637, thereby blocking I/R-induced mitochondrial division and protecting cardiac function [110]. Emagliflzin prevents mitochondrial division by activating AMPK, inhibiting the phosphorylation of Drp1 Ser-616, and increasing the phosphorylation of Drp1 Ser-637. In addition, Emagliflzin inhibits mitochondrial ROS production and subsequent oxidative stress, thereby preventing the senescence of cardiac microvascular endothelial cells. Consequently, the barrier function of cardiac microvascular endothelial cells is preserved, and the structure and function of diabetic cardiomyopathy are improved [111]. Similar to Emagliflzin, melatonin blunts Drp1-dependent mitochondrial fission by activating AMPKα, and subsequently downregulating Drp1 Ser-616 phosphorylation and upregulating Drp1 Ser-637 phosphorylation. By restoring the mitochondrial voltage-dependent anion channel (VDAC1), Melatonin interacts with hexokinase 2 (HK2), thereby hindering mitochondrial permeability transition pore opening and PINK1/Parkin activation, and ultimately preventing mitosis-mediated cell death and decreasing I/R damage [112]. No studies have shown that long-term melatonin use negatively affects Drp1. The same target is also activated by ophiopogonin D (OP-D), which stimulates Drp1 activity by increasing Drp1 phosphorylation at Ser-616 and decreasing phosphorylation at Ser-637; consequently, lipid accumulation and mitochondrial damage decrease in diabetic mouse cardiomyocytes [113]. In addition to targeting Drp1 phosphorylation, zinc has been demonstrated to induce mitochondrial mitosis by increasing Drp1 SUMOylation, thus leading to mitochondrial clearance, preventing ROS production during myocardial I/R injury, and preserving cardiomyocyte function [70]. By overexpression of CSE or use of exogenous sulfide, H_2S levels can be increased, and myocardial infarction and heart failure can be prevented [78, 79].

As a result of the discovery of many Drp1 PTMs and their loci, drugs targeting Drp1 PTMs have been developed. However, most of them function by regulating levels of Drp1 phosphorylation, whereas the functions and drugs of other PTMs remain unknown. We look forward to the development and clinical application of more drugs targeting Drp1 PTMs.

Discussion and Future Directions

Drp1 is a key protein in mitochondrial biological processes, and heart failure models in cardiac-specific Drp1 knockout mice have demonstrated the important role of Drp1 in maintaining normal cardiac function [114]. Regulation of Drp1 endogenous expression, Drp1 translocation to mitochondria, and Drp1-dependent mitosis is required to protect the heart against various stress-induced mitochondrial dysfunction and abnormal cardiac function. PTMs are important regulators of organism health and disease, and have great potential for clinical research, because they affect cellular biological activity by regulating protein levels and activity. This article reviewed the relationship between PTMs of the mitochondrial fission-dependent protein Drp1 and cardiac diseases. Dysregulation of numerous PTMs, such as SUMOylation, phosphorylation, acetylation, O-GlcNAcylation, and S-sulfhydration, of Drp1 cause cardiac dysfunction. These PTMs promote or

inhibit Drp1 activity, and their excessive activation or inhibition can lead to excessive mitochondrial fission or an inability of damaged mitochondria to perform autophagy, thereby leading to dysregulation of the dynamic balance of mitochondria, and ultimately to cell damage or even apoptosis. When mitochondrial dynamics is the therapeutic target under pathological conditions, controlling the degree of mitochondrial fusion and fission is critical, and "normal" levels should not be maintained under all conditions. For instance, the physiological upregulation of mitochondrial fission in cardiac muscle under stress is required for adaptation to heightened energy requirements, when inhibition of mitochondrial fission may lead to myocardial injury due to inadequate myocardial energy supply. Thus, the key issues associated with manipulating mitochondrial dynamics for the treatment of cardiovascular disease remain unresolved. The dynamic balance of mitochondria is critical, and how to maintain this balance is an important issue that must be addressed in the future.

Single PTMs are clearly essential in regulating protein structure-function relationships, but until recently, different modifications had gradually been discovered to interact with each other through cooperation or competition. This crosstalk among PTMs may serve as a novel mechanism of cellular regulation allowing rapid changes in various cellular functions. For example, in mouse embryonic fibroblasts, with the addition of multiple O-GlcNAcase inhibitors, ultra-high levels of O-GlcNacylation lead to reciprocal regulation of phosphorylation at more than 400 sites of multiple proteins (280 of which have decreased phosphorylation levels [115], and functional crosstalk between O-GlcNAc or phosphorylation of Drp1 has been identified. For example, high glucose treatment in cardiomyocytes induces O-GlcNacylation at Drp1 threonine 585 (T585) and threonine 586 (T586), thus decreasing phosphorylation at Drp1 Ser-637, promoting mitochondrial fission, and causing cardiac dysfunction [95]. Under high-fat diet conditions, acetylation of Drp1 at the K642 site may be indispensable for increasing Drp1 phosphorylation at the Ser-616 site, thereby promoting its mitochondrial translocation and GTPase activity [88]. This functional crosstalk among protein PTMs will be an important direction for future research and may provide a new feasible option for the treatment of cardiovascular diseases.

In summary, we conducted a comprehensive review of several specific Drp1 PTMs and demonstrated how these modifications modulate Drp1 and consequently elicit different outcomes. Continued research to identify potential targets may aid in designing more effective therapeutic strategies for cardiac diseases, and further exploration of various Drp1 modification-associated enzymes and loci should improve understanding of the function, mechanism, regulation, and therapeutic applications of Drp1. The PTMs of Drp1 have great potential for future research in the treatment of cardiac diseases, because the modifications themselves, and the crosstalk among them, can alter their activities and thus affect disease development. We believe that several outstanding questions require further research attention and consideration: How can the levels of Drp1 PTMs be targeted and regulated without affecting other proteins? How can the activity of Drp1 be precisely altered under different pathological conditions through PTMs? What are the specific mechanisms of functional crosstalk between different PTMs? What role does this crosstalk play in different cardiovascular diseases? Answering these questions should contribute to the development of new therapeutic agents to decrease cardiovascular disease morbidity and mortality.

Data Availability Statement

Data availability is not applicable to this article as no new data were created or analyzed in this study.

Author Contributions

Writing-original draft: YJJ and JJL. Writing-review and editing: YJJ, HZ, CY, and JJL. All authors have agreed on the final version to be published.

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Conflict of Interest

The authors declare they have no conflicts of interest.

REFERENCES

1. Vásquez-Trincado C, García-Carvajal I, Pennanen C, Parra V, Hill JA, Rothermel BA, et al. Mitochondrial dynamics, mitophagy and cardiovascular disease. J Physiol 2016;594:509–25.
2. Cogliati S, Enriquez JA, Scorrano L. Mitochondrial cristae: where beauty meets functionality. Trends Biochem Sci 2016;41:261–73.
3. Formosa LE, Ryan MT. Mitochondrial OXPHOS complex assembly lines. Nat Cell Biol 2018;20:511–3.
4. Friedman JR, Nunnari J. Mitochondrial form and function. Nature 2014;505:335–43.
5. Archer SL. Mitochondrial dynamics–mitochondrial fission and fusion in human diseases. N Engl J Med 2013;369:2236–51.
6. Smirnova E, Griparic L, Shurland DL, van der Bliek AM. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. Mol Biol Cell 2001;12:2245–56.
7. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 2008;27:433–46.
8. Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, et al. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J Cell Biol 2010;191:1367–80.
9. Cho B, Choi SY, Cho HM, Kim HJ, Sun W. Physiological and pathological significance of dynamin-related protein 1 (drp1)-dependent mitochondrial fission in the nervous system. Exp Neurobiol 2013;22:149–57.
10. Hu C, Huang Y, Li L. Drp1-dependent mitochondrial fission plays critical roles in physiological and pathological progresses in mammals. Int J Mol Sci 2017;18:144.
11. Kageyama Y, Hoshijima M, Seo K, Bedja D, Sysa-Shah P, Andrabi SA, et al. Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain. EMBO J 2014;33:2798–813.
12. Song M, Dorn GW. Mitoconfusion: noncanonical functioning of dynamism factors in static mitochondria of the heart. Cell Metab 2015;21:195–205.
13. Neuzil J, Widén C, Gellert N, Swettenham E, Zobalova R, Dong LF, et al. Mitochondria transmit apoptosis signalling in cardiomyocyte-like cells and isolated hearts exposed to experimental ischemia-reperfusion injury. Redox Rep 2007;12:148–62.
14. Biala AK, Kirshenbaum LA. The interplay between cell death signaling pathways in the heart. Trends Cardiovasc Med 2014;24:325–31.
15. Chang X, Li Y, Cai C, Wu F, He J, Zhang Y, et al. Mitochondrial quality control mechanisms as molecular targets in diabetic heart. Metabolism 2022;137:155313.
16. Sun D, Wang J, Toan S, Muid D, Li R, Chang X, et al. Molecular mechanisms of coronary microvascular endothelial dysfunction in diabetes mellitus: focus on mitochondrial quality surveillance. Angiogenesis 2022;25:307–29.
17. Liu W, Zhou C. Corticosterone reduces brain mitochondrial function and expression of mitofusin, BDNF in depression-like rodents regardless of exercise preconditioning. Psychoneuroendocrinology 2012;37:1057–70.
18. Ding H, Jiang N, Liu H, Liu X, Liu D, Zhao F, et al. Response of mitochondrial fusion and fission protein gene expression to exercise in rat skeletal muscle. Biochim Biophys Acta 2010;1800:250–56.
19. Sharp WW, Fang YH, Han M, Zhang HJ, Hong Z, Banathy A, et al. Dynamin-related protein 1 (Drp1)-mediated diastolic dysfunction in myocardial ischemia-reperfusion injury: therapeutic benefits of Drp1 inhibition to reduce mitochondrial fission. FASEB J 2014;28:316–26.
20. Ong S-B, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ, et al. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation 2010;121:2012–22.
21. Chang C-R, Manlandro CM, Arnoult D, Stadler J, Posey AE, Hill RB, et al. A lethal de novo mutation in the middle domain of the dynamin-related GTPase Drp1 impairs higher order assembly and mitochondrial division. J Biol Chem 2010;285:32494–503.
22. Fröhlich C, Grabiger S, Schwefel D, Faelber K, Rosenbaum E, Mears J, et al. Structural insights into oligomerization and mitochondrial remodelling of dynamin 1-like protein. EMBO J 2013;32:1280–92.
23. Ong S-B, Hausenloy DJ. Mitochondrial dynamics as a therapeutic target for treating cardiac diseases. Handb Exp Pharmacol 2017;240:251–79.
24. Chang C-R, Blackstone C. Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. Ann N Y Acad Sci 2010;1201:34–9.
25. Rosenbloom AB, Lee SH, To M, Lee A, Shin JY, Bustamante C. Optimized two-color super resolution imaging of Drp1 during mitochondrial fission with a slow-switching Dronpa variant. Proc Natl Acad Sci U S A 2014;111:13093–8.
26. Waterham HR, Koster J, van Roermund CW, Mooyer PA, Wanders RJ, Leonard JV. A lethal defect of mitochondrial and peroxisomal fission. N Engl J Med 2007;356:1736–41.
27. Wakabayashi J, Zhang Z, Wakabayashi N, Tamura Y, Fukaya M, Kensler TW, et al. The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. J Cell Biol 2009;186:805–16.
28. Ikeda Y, Shirakabe A, Maejima Y, Zhai P, Sciarretta S, Toli J, et al. Endogenous Drp1 mediates mitochondrial autophagy and protects the heart against energy stress. Circ Res 2015;116:264–78.

29. Venne AS, Kollipara L, Zahedi RP. The next level of complexity: crosstalk of posttranslational modifications. Proteomics 2014;14:513–24.

30. Kim YJ, Kang MJ, Kim E, Kweon TH, Park YS, Ji S, et al. O-GlcNAc stabilizes SMAD4 by inhibiting GSK-3β-mediated proteasomal degradation. Sci Rep 2020;10:19908.

31. Liu Y, Tavana O, Gu W. p53 modifications: exquisite decorations of the powerful guardian. J Mol Cell Biol 2019;11:564–77.

32. Hitosugi T, Chen J. Post-translational modifications and the Warburg effect. Oncogene 2014;33:4279–85.

33. Jin J-Y, Wei X-X, Zhi X-L, Wang X-H, Meng D. Drp1-dependent mitochondrial fission in cardiovascular disease. Acta Pharmacol Sin 2021;42:655–64.

34. Xiao H, Zhou H, Zeng G, Mao Z, Zeng J, Gao A. SUMOylation targeting mitophagy in cardiovascular diseases. J Mol Med (Berl) 2022;100:1511–38.

35. Qi Z, Huang Z, Xie F, Chen L. Dynamin-related protein 1: a critical protein in the pathogenesis of neural system dysfunctions and neurodegenerative diseases. J Cell Physiol 2019;234:10032–46.

36. Quiles JM, Gustafsson ÅB. The role of mitochondrial fission in cardiovascular health and disease. Nat Rev Cardiol 2022;19:723–36.

37. Huang C-Y, Lee FL, Peng SF, Lin KH, Chen RJ, Ho TJ, et al. HSF1 phosphorylation by ERK/GSK3 suppresses RNF126 to sustain IGF-IIR expression for hypertension-induced cardiomyocyte hypertrophy. J Cell Physiol 2018;233:979–89.

38. Shou J, Huo Y. PINK1 phosphorylates Drp1S616 to improve mitochondrial fission and inhibit the progression of hypertension-induced HFpEF. Int J Mol Sci 2022;23:11934.

39. Huang C-Y, Lai CH, Kuo CH, Chiang SF, Pai PY, Lin JY, et al. Inhibition of ERK-Drp1 signaling and mitochondria fragmentation alleviates IGF-IIR-induced mitochondria dysfunction during heart failure. J Mol Cell Cardiol 2018;122:58–68.

40. Zhang Y, Zhang L, Zhang Y, Fan X, Yang W, Yu B, et al. YiQiFuMai powder injection attenuates coronary artery ligation-induced heart failure through improving mitochondrial function via regulating ROS generation and CaMKII signaling pathways. Front Pharmacol 2019;10:381.

41. Hill JA, Olson EN. Cardiac plasticity. N Engl J Med 2008;358:1370–80.

42. Chang Y-W, Chang YT, Wang Q, Lin JJ, Chen YJ, Chen CC. Quantitative phosphoproteomic study of pressure-overloaded mouse heart reveals dynamin-related protein 1 as a modulator of cardiac hypertrophy. Mol Cell Proteomics 2013;12:3094–107.

43. Jong CJ, Yeung J, Tseung E, Karmazyn M. Leptin-induced cardiomyocyte hypertrophy is associated with enhanced mitochondrial fission. Mol Cell Biochem 2019;454:33–44.

44. Pennanen C, Parra V, López-Crisosto C, Morales PE, Del Campo A, Gutierrez T, et al. Mitochondrial fission is required for cardiomyocyte hypertrophy mediated by a Ca2+-calcineurin signaling pathway. J Cell Sci 2014;127:2659–71.

45. Cheng W, Yang S, Li X, Liang F, Zhou R, Wang H, et al. Low doses of BPA induced abnormal mitochondrial fission and hypertrophy in human embryonic stem cell-derived cardiomyocytes via the calcineurin-DRP1 signaling pathway: A comparison between XX and XY cardiomyocytes. Toxicol Appl Pharmacol 2020;388:114850.

46. Zhen C, Liu H, Gao L, Tong Y, He C. Signal transducer and transcriptional activation 1 protects against pressure overload-induced cardiac hypertrophy. FASEB J 2021;35:e21240.

47. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev 2008;88:581–609.

48. Nishimura A, Shimauchi T, Tanaka T, Shimoda K, Toyama T, Kitajima N, et al. Hypoxia-induced interaction of filamin with Drp1 causes mitochondrial hyperfission-associated myocardial senescence. Sci Signal 2018;11:eaat5185.

49. Zepeda R, Kuzmicic J, Parra V, Troncoso R, Pennanen C, Riquelme JA, et al. Drp1 loss-of-function reduces cardiomyocyte oxygen dependence protecting the heart from ischemia-reperfusion injury. J Cardiovasc Pharmacol 2014;63:477–87.

50. Chang X, Toan S, Li R, Zhou H. Therapeutic strategies in ischemic cardiomyopathy: Focus on mitochondrial quality surveillance. EBioMedicine 2022;84:104260.

51. Taylor D, Germano J, Song Y, Hadj-Moussa H, Marek-Iannucci S, Dhanji R, et al. Hypothermia promotes mitochondrial elongation in cardiac cells via inhibition of Drp1. Cryobiology 2021;102:42–55.

52. Huang X, Yan Y, Zheng W, Ma Y, Wang X, Gong W, et al. Secreted frizzled-related protein 5 protects against cardiac rupture and improves cardiac function through inhibiting mitochondrial dysfunction. Front Cardiovasc Med 2021;8:682409.

53. Liu D, Zou S, Li G, Zhang Q, Chen C, Li C, et al. Downregulation of uncoupling protein 2(UCP2) mediated by microRNA-762 confers cardioprotection and participates in the regulation of dynamic mitochondrial homeostasis of dynamin related protein 1 (DRP1) after myocardial infarction in mice. Front Cardiovasc Med 2021;8:764064.

54. Liu J, Yan W, Zhao X, Jia Q, Wang J, Zhang H, et al. Sirt3 attenuates post-infarction cardiac injury via inhibiting mitochondrial fission and normalization of AMPK-Drp1 pathways. Cell Signal 2019;53:1–13.

55. Davidson SM, Ferdinandy P, Andreadou I, Bøtker HE, Heusch G, Ibáñez B, et al. Multitarget strategies to reduce myocardial ischemia/reperfusion injury: JACC review topic of the week. J Am Coll Cardiol 2019;73:89–99.

56. Chang X, Lochner A, Wang HH, Wang S, Zhu H, Ren J, et al. Coronary microvascular injury in myocardial infarction: perception and knowledge for mitochondrial quality control. Theranostics 2021;11:6766–85.

57. Zhu H, Tan Y, Du W, Li Y, Toan S, Mui D, et al. Phosphoglycerate mutase 5 exacerbates cardiac ischemia-reperfusion injury through disrupting mitochondrial quality control. Redox Biol 2021;38:101777.
58. She L, Tu H, Zhang YZ, Tang LJ, Li NS, Ma QL, et al. Inhibition of phosphoglycerate mutase 5 reduces necroptosis in rat hearts following ischemia/reperfusion through suppression of dynamin-related protein 1. Cardiovasc Drugs Ther 2019;33:13–23.
59. Du J, Li H, Song J, Wang T, Dong Y, Zhan A, et al. AMPK activation alleviates myocardial ischemia-reperfusion injury by regulating Drp1-mediated mitochondrial dynamics. Front Pharmacol 2022;13:862204.
60. Hinderer S, Schenke-Layland K. Cardiac fibrosis - A short review of causes and therapeutic strategies. Adv Drug Deliv Rev 2019; 146:77–82.
61. Gao Q-Y, Zhang HF, Tao J, Chen ZT, Liu CY, Liu WH, et al. Mitochondrial fission and mitophagy reciprocally orchestrate cardiac fibroblasts activation. Front Cell Dev Biol 2020;8:629397.
62. Tseng H-C, Lin C-C, Hsiao L-D, Yang C-M. Lysophosphatidylcholine-induced mitochondrial fission contributes to collagen production in human cardiac fibroblasts. J Lipid Res 2019;60:1573–89.
63. Adaniya SM, O-Uchi J, Cypress MW, Kusakari Y, Jhun BS. Posttranslational modifications of mitochondrial fission and fusion proteins in cardiac physiology and pathophysiology. Am J Physiol Cell Physiol 2019;316:C583–604.
64. Shimizu Y, Lambert JP, Nicholson CK, Kim JJ, Wolfson DW, Cho HC, et al. DJ-1 protects the heart against ischemia-reperfusion injury by regulating mitochondrial fission. J Mol Cell Cardiol 2016;97:56–66.
65. Karhausen J, Bernstock JD, Johnson KR, Sheng H, Ma Q, Shen Y, et al. Ubc9 overexpression and SUMO1 deficiency blunt inflammation after intestinal ischemia/reperfusion. Lab Invest 2018;98:799–813.
66. Boyer MJ, Eguchi S. A cytoskeletal anchor connects ischemic mitochondrial fission to myocardial senescence. Sci Signal 2018;11:eaav3267.
67. Guo C, Wilkinson KA, Evans AJ, Rubin PP, Henley JM. SENP3-mediated deSUMOylation of Drp1 facilitates interaction with Mff to promote cell death. Sci Rep 2017;7:43811.
68. Fu J, Yu HM, Chiu SY, Mirando AJ, Maruyama EO, Cheng JG, et al. Disruption of SUMO-specific protease 2 induces mitochondria mediated neurodegeneration. PLoS Genet 2014;10:e1004579.
69. Zunino R, Schauss A, Rippstein P, Andrade-Navarro M, McBride HM. The SUMO protease SENP5 is required to maintain mitochondrial morphology and function. J Cell Sci 2007;120:1178–88.
70. Bian X, Xu J, Zhao H, Zheng Q, Xiao X, Ma X, et al. Zinc-induced SUMOylation of dynamin-related protein 1 protects the heart against ischemia-reperfusion injury. Oxid Med Cell Longev 2019;2019:1232146.
71. Montagna C, Cirotti C, Rizza S, Filomeni G. When S-nitrosylation gets to mitochondria: from signaling to age-related diseases. Antioxid Redox Signal 2020;32: 884–905.
72. Bossy B, Petrilli A, Klinglmayr E, Chen J, Lütz-Meindl U, Knott AB, et al. S-Nitrosylation of DRP1 does not affect enzymatic activity and is not specific to Alzheimer's disease. J Alzheimers Dis 2010;20(Suppl 2): S513–26.
73. Kayki-Mutlu G, Koch WJ. Nitric oxide and S-nitrosylation in cardiac regulation: G protein-coupled receptor kinase-2 and β-arrestins as targets. Int J Mol Sci 2021;22:521.
74. Paul BD, Snyder SH. Protein sulfhydration. Methods Enzymol 2015;555:79–90.
75. Barr LA, Calvert JW. Discoveries of hydrogen sulfide as a novel cardiovascular therapeutic. Circ J 2014;78:2111–8.
76. Cheung SH, Lau JYW. Hydrogen sulfide mediates athero-protection against oxidative stress via S-sulfhydration. PLoS One 2018;13:e0194176.
77. Li Z, Polhemus DJ, Lefer DJ. Evolution of hydrogen sulfide therapeutics to treat cardiovascular disease. Circ Res 2018;123:590–600.
78. Kondo K, Bhushan S, King AL, Prabhu SD, Hamid T, Koenig S, et al. H S protects against pressure overload-induced heart failure via upregulation of endothelial nitric oxide synthase. Circulation 2013;127:1116–27.
79. Kang J, Li Z, Organ CL, Park CM, Yang CT, Pacheco A, et al. pH-controlled hydrogen sulfide release for myocardial ischemia-reperfusion injury. J Am Chem Soc 2016;138:6336–9.
80. Wu D, Tan B, Sun Y, Hu Q. Cystathionine γ lyase S-sulfhydrates Drp1 to ameliorate heart dysfunction. Redox Biol 2022;58:102519.
81. Baeza J, Smallegan MJ, Denu JM. Mechanisms and dynamics of protein acetylation in mitochondria. Trends Biochem Sci 2016;41:231–44.
82. Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, et al. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 1996;84:843–51.
83. Taunton J, Hassig CA, Schreiber SL. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science 1996;272:408–11.
84. Horton JL, Martin OJ, Lai L, Riley NM, Richards AL, Vega RB, et al. Mitochondrial protein hyperacetylation in the failing heart. JCI Insight 2016;2:e84897.
85. Lai L, Leone TC, Keller MP, Martin OJ, Broman AT, Nigro J, et al. Energy metabolic reprogramming in the hypertrophied and early stage failing heart: a multisystems approach. Circ Heart Fail 2014;7:1022–31.
86. Lee CF, Chavez JD, Garcia-Menendez L, Choi Y, Roe ND, Chiao YA, et al. Normalization of NAD+ redox balance as a therapy for heart failure. Circulation 2016;134:883–94.

87. Bertero E, Maack C. Metabolic remodelling in heart failure. Nat Rev Cardiol 2018;15:457–70.

88. Hu Q, Zhang H, Gutiérrez Cortés N, Wu D, Wang P, Zhang J, et al. Increased Drp1 acetylation by lipid overload induces cardiomyocyte death and heart dysfunction. Circ Res 2020;126:456–70.

89. Kreppel LK, Blomberg MA, Hart GW. Dynamic glycosylation of nuclear and cytosolic proteins. Cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. J Biol Chem 1997;272:9308–15.

90. Gao Y, Wells L, Comer FI, Parker GJ, Hart GW. Dynamic O-glycosylation of nuclear and cytosolic proteins: cloning and characterization of a neutral, cytosolic beta-N-acetylglucosaminidase from human brain. J Biol Chem 2001;276:9838–45.

91. Yang X, Ongusaha PP, Miles PD, Havstad JC, Zhang F, So WV, et al. Phosphoinositide signalling links O-GlcNAc transferase to insulin resistance. Nature 2008;451:964–9.

92. Yi W, Clark PM, Mason DE, Keenan MC, Hill C, Goddard WA 3rd, et al. Phosphofructokinase 1 glycosylation regulates cell growth and metabolism. Science 2012;337:975–80.

93. Yuzwa SA, Shan X, Macauley MS, Clark T, Skorobogatko Y, Vosseller K. Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. Nat Chem Biol 2012;8:393–9.

94. Watson LJ, Facundo HT, Ngoh GA, Ameen M, Brainard RE, Lemma KM, et al. O-linked β-N-acetylglucosamine transferase is indispensable in the failing heart. Proc Natl Acad Sci U S A 2010;107:17797–802.

95. Gawlowski T, Suarez J, Scott B, Torres-Gonzalez M, Wang H, Schwappacher R, et al. Modulation of dynamin-related protein 1 (DRP1) function by increased O-linked-β-N-acetylglucosamine modification (O-GlcNAc) in cardiac myocytes. J Biol Chem 2012;287:30024–34.

96. Zhao J, Dong L, Huo T, Cheng J, Li X, Huangfu X, et al. O-GlcNAc transferase (OGT) protects cerebral neurons from death during ischemia/reperfusion (I/R) injury by modulating Drp1 in mice. Neuromolecular Med 2022;24:299–310.

97. Pagliuso A, Cossart P, Stavru F. The ever-growing complexity of the mitochondrial fission machinery. Cell Mol Life Sci 2018;75:355–74.

98. Damgaard RB. The ubiquitin system: from cell signalling to disease biology and new therapeutic opportunities. Cell Death Differ 2021;28:423–6.

99. Yonashiro R, Ishido S, Kyo S, Fukuda T, Goto E, Matsuki Y, et al. A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. EMBO J 2006;25:3618–26.

100. Wang H, Song P, Du L, Tian W, Yue W, Liu M, et al. Parkin ubiquitinates Drp1 for proteasome-dependent degradation: implication of dysregulated mitochondrial dynamics in Parkinson disease. J Biol Chem 2011;286:11649–58.

101. Nagashima S, Tokuyama T, Yonashiro R, Inatome R, Yanagi S. Roles of mitochondrial ubiquitin ligase MITOL/MARCH5 in mitochondrial dynamics and diseases. J Biochem 2014;155:273–9.

102. Karbowski M, Neutzner A, Youle RJ. The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division. J Cell Biol 2007;178:71–84.

103. Cherok E, Xu S, Li S, Das S, Meltzer WA, Zalzman M, et al. Novel regulatory roles of Mff and Drp1 in E3 ubiquitin ligase MARCH5-dependent degradation of MiD49 and Mcl1 and control of mitochondrial dynamics. Mol Biol Cell 2017;28:396–410.

104. Das T, Yount JS, Hang HC. Protein S-palmitoylation in immunity. Open Biol 2021;11:200411.

105. Tang M, Lu L, Huang Z, Chen L. Palmitoylation signaling: a novel mechanism of mitochondria dynamics and diverse pathologies. Acta Biochim Biophys Sin (Shanghai) 2018;50:831–3.

106. Napoli E, Song G, Liu S, Espejo A, Perez CJ, Benavides F, et al. Zdhhc13-dependent Drp1 S-palmitoylation impacts brain bioenergetics, anxiety, coordination and motor skills. Sci Rep 2017;7:12796.

107. Xia Y, Chen Z, Chen A, Fu M, Dong Z, Hu K, et al. LCZ696 improves cardiac function via alleviating Drp1-mediated mitochondrial dysfunction in mice with doxorubicin-induced dilated cardiomyopathy. J Mol Cell Cardiol 2017;108:138–48.

108. Deng Y, Li S, Chen Z, Wang W, Geng B, Cai J. Mdivi-1, a mitochondrial fission inhibitor, reduces angiotensin-II- induced hypertension by mediating VSMC phenotypic switch. Biomed Pharmacother 2021;140:111689.

109. Nan J, Zhu W, Rahman MS, Liu M, Li D, Su S, et al. Molecular regulation of mitochondrial dynamics in cardiac disease. Biochim Biophys Acta Mol Cell Res 2017;1864:1260–73.

110. Chen L, Chen XY, Wang QL, Yang SJ, Zhou H, Ding LS, et al. Astragaloside IV derivative (LS-102) alleviated myocardial ischemia reperfusion injury by inhibiting Drp1Ser616 phosphorylation-mediated mitochondrial fission. Front Pharmacol 2020;11:1083.

111. Zhou H, Wang S, Zhu P, Hu S, Chen Y, Ren J. Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission. Redox Biol 2018;15:335–46.

112. Zhou H, Zhang Y, Hu S, Shi C, Zhu P, Ma Q, et al. Melatonin protects cardiac microvasculature against ischemia/reperfusion injury via suppression of mitochondrial

fission-VDAC1-HK2-mPTP-mitophagy axis. J Pineal Res 2017;63:e12413.

113. Li W, Ji L, Tian J, Tang W, Shan X, Zhao P, et al. Ophiopogonin D alleviates diabetic myocardial injuries by regulating mitochondrial dynamics. J Ethnopharmacol 2021;271:113853.

114. Song M, Mihara K, Chen Y, Scorrano L, Dorn GW. Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts. Cell Metab 2015;21:273–86.

115. Liddy KA, White MY, Cordwell SJ. Functional decorations: post-translational modifications and heart disease delineated by targeted proteomics. Genome Med 2013;5:20.