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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, especially in undivided cells with high energy requirements such as cardiomyocytes, where they change their shape and position through the activity of mitochondrial fission proteins and mitochondrial fusion proteins. These are significant regulatory mechanisms for cardiomyocyte energy supply and normal function. In mitochondrial fission, Dynamin-Related Protein 1 (Drp1) is involved in the separation and degradation of damaged mitochondria, which accurately regulates the renewal and number of mitochondria. Recent studies have shown that there are 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 different signaling

pathways by altering its activity, stability, and subcellular localization. This paper provides an overview of the relationship between Drp1 PTMs and cardiovascular diseases such as heart failure, myocardial infarction, and myocardial ischemia-reperfusion, as well as how these modifications can be targeted and regulated to help guide cardiovascular disease treatment.

Introduction

Mitochondria, which provide energy for cellular life activities, are abundant in muscle tissue, especially in the heart, and they have an important impact 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 to maintain contractile function[2]. The morphology, number and size of mitochondria are constantly changing 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, accompanied by mitochondrial biogenesis and mitochondrial-specific mitophagy. Mitochondrial dynamics was formed to study its specific mechanism[3-5]. An increasing number of studies have shown that mitochondrial dynamics is closely related to mitochondrial function. Disruption of the dynamic balance of mitochondrial fission and fusion leads to structural alterations and dysfunction; at the same time, mitochondrial fission and fusion are early responses to mitochondrial dysfunction that enhance their viability and maintain normal form and function[6,7].

Mitochondrial dynamics is regulated by a variety of proteins through change in their number and activity. Among them, mammalian dynamin-related 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 mainly through numerous mechanisms such as effect on cellular energy metabolism, regulation of intracellular calcium levels, and 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, reduced aerobic respiration, and inadequate cellular energy supply[12]. In addition, 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 not only reflected at the level of its protein, but its activity is also strictly regulated by post-translational modifications (PTMs)[17,18]. For example, during reperfusion, Drp1 Ser637 is activated by increased dephosphorylation, which in turn leads to mitochondrial translocation, resulting in increased undesirable mitochondrial fissions[19], whereas the calcineurin inhibitor FK506 prevents dephosphorylation of Drp1 Ser637 and protects cardiac function during I/R [20]. We will 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 of DrP1 and its function

Drp1 is a GTP binding protein that belongs to 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 the C-terminal GTPase effector domain (GED)[21]. Crystal structure studies have shown that their VDs act as hinges by forming T-dimers or tetramers and effectively binding to 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; then, Drp1 GTPase is activated, which leads to the remodeling of the mitochondrial membrane and its contraction; and finally,

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mitochondrial fission is induced to produce 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) (Fig. 1). In contrast, the endoplasmic reticulum can also play a role in assisting in the recruitment of Drp1 by transferring calcium ions into the mitochondria[25]. Drp1 is inextricably linked to disease development, and in humans, fission-defective Drp1 mutations are associated with fatal microcephaly[26]; mouse embryonic cells with Drp1 knockout have dramatically downregulated ATP levels and fail to survive[27]; similarly, in Neonatal Rat Ventricular Myocytes (NRVMs), deletion of Drp1 leads to the accumulation of damaged mitochondria, decreases intracellular ATP, and leads to apoptosis[28]. In addition to Drp1 protein levels, the role of Drp1 depends on the dynamic balance of its activity, a process regulated by various mechanisms, such as ROS, CDK1, PKCδ and calcineurin (CaN). By regulating its activity to maintain normal mitochondrial fission, Drp1 can effectively control the relevant disease process[7]. At present, how to precisely alter Drp1 activity to achieve therapeutic effects remains to be investigated.

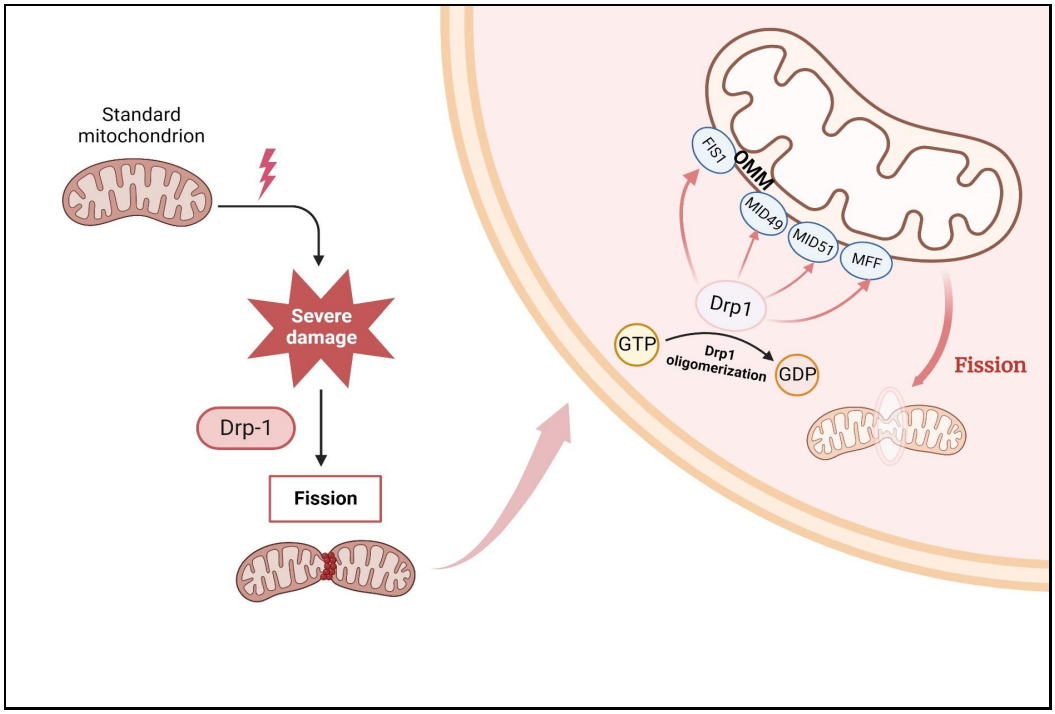

Figure 1: Cytosolic dynamin related protein 1 (Drp1) monomers are recruited to the

outer mitochondrial membrane by the adaptor proteins mitochondrial fission 1 protein (FIS1), mitochondrial dynamics protein 49 (MID49), MID51 and mitochondrial fission factor (MFF), which facilitates the formation of helical ring Drp1 oligomers. GTP hydrolysis by Drp1 stimulates outer mito chondrial 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 is created with BioRender.com）

PTMs of Drp1 in the heart

PTMs, covalent processing events that change the biochemical properties of a protein by proteolytic cleavage and adding a modifying group to one or more amino acids under the catalysis of enzymes, play a key role in many biological processes. To date, more than 450 protein PTMs have been identified, including phosphorylation, ubiquitination, SUMOylation, acetylation, methylation and O-GlcNAcylation. These PTMs are capable of altering the activity, stability, protein interactions and intracellular localization of the target protein[29]. Most PTMs are reversible and quantitatively alterable, and multiple PTMs can also regulate each other. This regulation can be competitive or facilitative, as in the case of the Recombinant Mothers Against Decapentaplegic Homolog 4 (SMAD4) protein where phosphorylation and O-GlcNAcylation compete with each other for modification sites[30]; ubiquitination and acetylation of the cancer suppressor gene P53 can be mutually blocked at certain specific sites, while phosphorylation can promote or inhibit acetylation, which is site-specific[31]. Furthermore, different sites of the same modification may have different effects on protein function. Due to their reversible nature of precise regulation, cells can use them as "switches" for rapid changes in cellular state[32].

In normal cells, Drp1 is modified by various PTMs that can influence specific functions of Drp1 to maintain cellular environmental homeostasis in a given

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environment. In addition, crosstalk between different PTMs on Drp1 can be regulated to allow cells to adapt to various changes in different environments[33]. Abnormalities in PTMs can lead to abnormal activity of Drp1, which can affect cellular processes such as mitochondrial fission, energy supply, and thus induce or exacerbate heart disease[34]. PTMs provide a new perspective on the regulation of Drp1 protein levels/activity with the presence of numerous PTMs targets (Figure 2). In this paper, we reviewed the function of such PTMs as SUMOylation, phosphorylation, acetylation, O-GlcNAcylation, and S-sulfhydration of regulating Drp1 and their correlation with cardiac diseases, and further highlighted that PTMs targeting Drp1 may have great potential in future heart disease-related therapies.

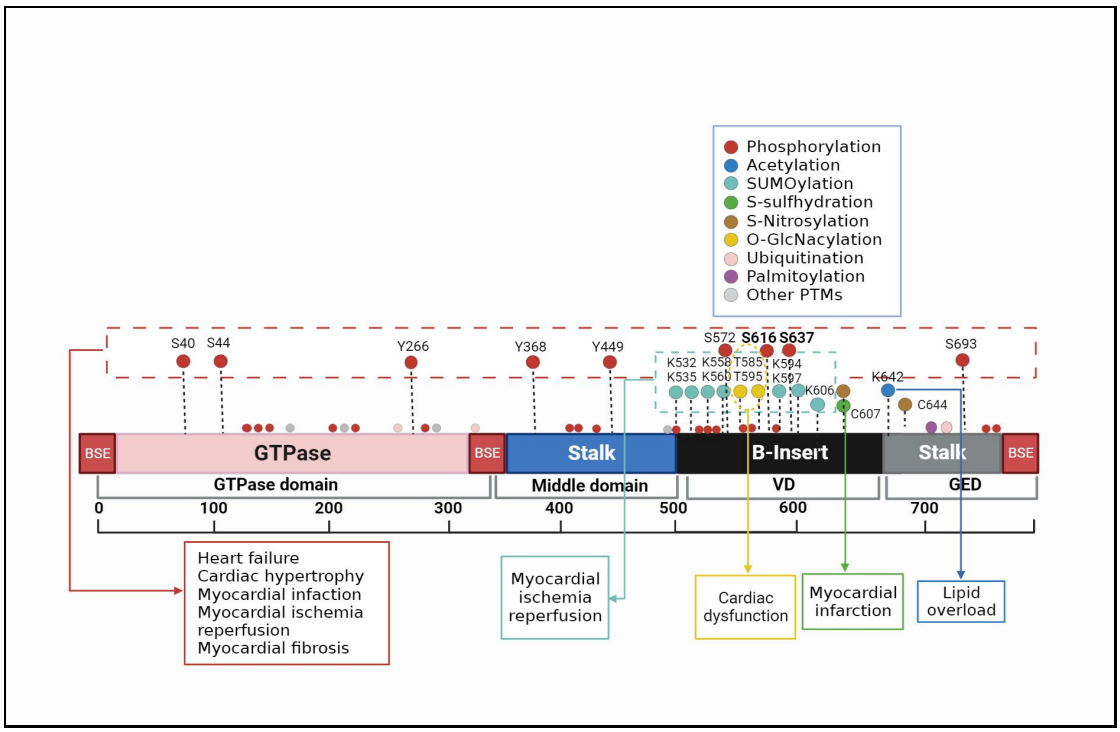

Figure 2: Chart showing Drp1 structure, PTMs distribution and related cardiac diseases. The domain structure of Drp1 includes the N-terminal GTPase domain, middle domain, B-insert, and the C-terminal GTPase effector domain. Amino acid residues without site information are indicated only from proteomics and mass spectrometry data. Phosphorylation sites include: S40, S44, Y266, Y368, Y449, S572, S616, S637, S693, related cardiac diseases: Heart failure, Cardiac hypertrophy, Myocardial infarction, Myocardial ischemia reperfusion Myocardial fibrosis;

acetylation sites include: K642, related heart disease: Lipid overload; SUMOylation sites: K532, K535, K558, K560, K594, K597, K606, related heart disease: Myocardial ischemia reperfusion; S-sulfhydration sites include: C607, C644, related cardiac disease: Cardiac infarction; O-GlcNacylation sites include: T585, T595, related cardiac disease: Cardiac dysfunction S-Nitrosylation sites include: C607; BSE, bundle signaling element; GED, GTPase effector domain; VD, variable domain.（figure is created with BioRender.com）

Phosphorylation

Phosphorylation is the most prominent and extensively studied PTMs of Drp1. Depending on the 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, Ser592, Ser-656, Ser-616, Ser-637, and Ser-693[35]. Among them, Ser616 and Ser637 (corresponding to mouse Ser579 and Ser600, respectively) are the two most studied phosphorylation sites involved in the regulation of mitochondrial fission[36]. We will discuss the role of Drp1 phosphorylation in different cardiac disorders in the following section.

Numerous studies have shown that mitochondrial function has an important impact on the development of heart failure. Drp1 phosphorylation is indispensable for myocardial energy supply. For example, fibronectin increases the phosphorylation level of the Drp1 S616 site via FAK-ERK1/2-Drp1, increasing the oxygen consumption rate and ATP content of NRVMS. Inhibition of the FAK-ERK1/2-Drp1 pathway would result in a shortage of cellular energy, leading to a decrease in 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 S616 and promotes mitochondrial fission, thereby slowing the progression of hypertension (hypertension)-induced HFpEF[38]. However, it has also been demonstrated that Drp1 hyperphosphorylation is a detrimental factor in heart failure.

For instance, insulin-like growth factor II receptor (IGF-IIR) mediates extracellular regulated protein kinases (ERK) activation, resulting in hyperphosphorylation of the Drp1 S616 site and its translocation to mitochondria. Accordingly, mitochondrial fission and dysfunction enhances the ability of the Rab9-dependent autophagosome to recognize and engulf damaged mitochondria, ultimately reducing cardiomyocyte viability[39]. Blocking IGF-IIR signaling can effectively inhibit cardiomyocyte hypertrophy and apoptosis[37], and inhibiting Drp1 activity by regulating Drp1 phosphorylation levels can attenuate the loss of Mitochondria Transmembrane Potential and decrease in cell survival caused by IGF-IIR activation[39]. YiQiFuMai Powder Injection significantly reduced coronary artery ligation (CAL)-induced heart failure by improving mitochondrial morphology, increasing mitochondrial membrane potential, reducing Drp1 phosphorylation, and protecting mitochondrial function [40]. The above experiments indicate that the phosphorylation of Drp1 is closely related to the development of heart failure, and the regulation of Drp1 phosphorylation levels under specific conditions holds significance for the protection of cardiac function.

Hypertension or acute coarctation of aorta that results in pressure overload in the left ventricle (LV) can cause pathological myocardial hypertrophy. Due to compensation, abnormal metabolic, structural, and functional signals are generated, which in turn result in abnormal cardiac function[41]. Therefore, inhibiting myocardial hypertrophy is a hot topic of current research. Drp1 Ser622 can be phosphorylated by PKC-δ. in rat cardiomyocytes, leading to myocardial hypertrophy[42]. Leptin-induced cardiomyocyte hypertrophy is associated with calcium-regulated neurophosphatase-mediated Drp1 Ser637 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 Ser637[44]. Low doses of bisphenol A (BPA) are capable of causing myocardial hypertrophy through calcium homeostasis via the calcium-regulated neurophosphatase-Drp1 Ser637 signaling pathway[45]. In spite of the fact that these studies appear to indicate that elevated levels of Drp1 phosphorylation promote 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, and thus inhibit myocardial hypertrophy[46]. According to the above studies, Drp1 phosphorylation promotes myocardial hypertrophy in myocardial structure, whereas Drp1 phosphorylation inhibits myocardial hypertrophy in energy supply and thus inhibits myocardial hypertrophy. Due to the specific roles played by Drp1 phosphorylation in different pathways, there may be complex functional regulatory crosstalk, and regulating Drp1 phosphorylation levels may be a significant 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, resulting in significant mitochondrial fragmentation and cardiac dysfunction[48]. By inhibiting Drp1 activity, mitochondrial metabolic disturbances, and fragmentation can be reduced, protecting the heart from the adverse effects of myocardial infarction[49,50]. Hypothermia promotes myocardial mitochondrial lengthening by inhibiting Drp1 S616 phosphorylation, which decreases oxygen consumption and thereby reduces ischemic injury[51]. Secreted frizzled-related protein 5 (Sfrp5) is considered a protective regulatory protein in coronary heart disease, and Sfrp5 reduces Drp1 S616 phosphorylation levels and significantly reduced infarct size[52]. Similar to Sfrp5 action, uncoupling protein 2 (UCP2) overexpression during myocardial infarction inhibits Drp1 S616 phosphorylation, which in turn reduces apoptosis and improves cardiac function[53]. By disrupting mitochondrial fission and activating AMPK, Sirtuin 3 (SIRT3) can delay myocardial injury after myocardial infarction by promoting Drp1 dephosphorylation and Drp1 phosphorylation[54]. Based on the above studies, it is demonstrated that regulating Drp1 phosphorylation levels is capable of substantially enhancing cardiomyocyte survival following myocardial infarction.

Following a myocardial infarction, emergency open occluded coronary arteries are performed in order to restore blood flow to the ischemic tissues. In spite of this, myocardial tissue is destroyed within the first few minutes of reperfusion, resulting in a sustained cardiac injury. This occurrence referred to as ischemia-reperfusion (I/R)

injury is responsible for the expansion of the infarcted region[55]. A growing body of evidence indicates that excessive mitochondrial fission can contribute to cardiomyocyte death following I/R[56]. During ischemia and reperfusion, mitochondrial fission is activated, resulting in high mitochondrial production of reactive oxygen species (ROS), calcium overload, and over-opening of mitochondrial permeability transition pores (MPTPs)[36]. In mice models of myocardial I/R injury, elevated levels of Phosphoglycerate Mutase 5 (PGAM5) protein cause myocardial dephosphorylation of Drp1 S637 and increased phosphorylation of Drp1 S616, which may lead to mitochondrial fragmentation and dysfunction. This is an early manifestation of myocardial I/R injury symptoms, and PGAM5 knockdown inhibits Drp1 S637 dephosphorylation in mice. While PGAM5 deletion does not affect Drp1 S616 phosphorylation, it still partially inhibits mitochondrial fragmentation and reduces I/R injury[57,58]. In mice models of I/R injury, the AMP-activated Protein Kinase (AMPK) agonist AICAR ameliorated isolated cardiac function and reduced arrhythmia incidence and myocardial infarct size by increasing AMPK activity, which in turn decreased Drp1 phosphorylation at Ser616 and increased Drp1 phosphorylation at Ser637[59]. Based on the results of these studies, it may be possible to protect mitochondria and cardiomyocytes under I/R injury by modulating Drp1 phosphorylation.

In wound healing following 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, ultimately leading to cardiac dysfunction[60]. Transforming growth factor-β (TGF-β) stimulates fibroblast proliferation, migration, and extracellular matrix synthesis, leading to myocardial fibrosis; inhibition of Drp1 suppresses these TGF-β-stimulated processes[61], which provides an opportunity to modulate myocardial fibrosis through Drp1. It has been shown that 3-sn-lysophosphatidylcholine (LPC)-induced protein kinase C (PKC) interacts with Drp1 and phosphorylates Drp1 Ser-616, thereby enhancing mitochondrial fission and depolarization mediated by Drp1. By inhibiting this pathway, fibroblast activation and collagen accumulation are

reduced in the heart, resulting in a reduction of fibrosis[62]. Consequently, inhibiting Drp1 phosphorylation may reduce excessive activation of fibroblast mitochondrial fission under cardiac stress and reduce heart remodeling and fibrosis.

SUMOylation

SUMO consists of a small ubiquitin-like modifier that is attached to the substrate protein by an enzyme linked reaction. SUMOylation entails a reversible and dynamic modification of lysine residues in a substrate protein, e.g., Lys-532, Lys -535, Lys-594, Lys-608, Lys-606, Lys-558, Lys-568, and Lys-597)[63], often altering proteins' subcellular localization or protecting them from ubiquitin-triggered damage. In the last decade, many studies have shown that SUMOylation contributes to cardiac perfusion[64,65]. Furthermore, SUMOylation is involved in the regulation of Drp1 activity[66]. Current understanding dictates that SUMO2/3 can SUMOylate 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 furnishes a defense against myocardial ischemia/reperfusion injury caused by zinc. Zinc-mediated Drp1 SUMOylation enhances mitophagy during reperfusion, thereby reducing ROS and myocardial damage[70]. As indicated by the findings, Drp1 SUMOylation exerts a considerable influence on cardiac function; however, the means by which it may meet clinical therapeutic standards is yet to be ascertained

S-nitrosylation

Cysteine plays a vital 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, resulting in the formation of protein S-nitrosylation, a redox switch involved in a number of 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 boost the formation of Drp1 oligomers, enhance GTPase activity, and alter protein conformation, inducing mitochondrial disruption, synaptic damage and bioenergetic failure, leading to the exacerbation of neurodegenerative diseases such as Alzheimer's and Huntington's disease[35]. However, it has been demonstrated that S-nitrosylation of Drp1 has no direct effect on Drp1 activity, but rather Drp1 C644 S-nitrosylation leads to increased mitochondrial fission by promoting Drp1 Ser616 phosphorylation[72]. It is evident from these findings that aberrant NO production leads to mitochondrial and synaptic dysfunction. In the cardiovascular system, there are several proteins that undergo S-nitrosylation, including calcium homeostasis-related proteins, mitochondrial proteins, hemoglobin, and myosin and ion channels that regulate contractility are targets of endogenous and exogenous NO S-nitrosylation[73]. Nevertheless, there is still controversy 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 (H2S) is a product of cardiac cystathionine γ-lyase (CSE) metabolism and a known biological active gas substance[74]. The physiological properties of hydrogen sulfide include modulation of vasodilation, anti-inflammatory, antioxidant, and angiogenesis[75]. During cardiac injury, endogenous production of hydrogen sulfide is reduced, while insufficient production results in heart disease[76]. H2S is believed to have anti-atherogenic properties that prevent atherosclerosis[77]. Studies have shown that cardiac-specific CSE gene overexpression mice maintained

cardiac structure and function following transverse aortic constriction (TAC)[78]. Additionally, exogenous sulfide treatment to increase H2S levels also demonstrated effective myocardial protection in mice models of MI/R injury[79]. Several studies have demonstrated that S-sulfhydration of H2S competes directly with S-nitrosylation of NO for binding the cysteine 607 of Drp1. Drp1 S-sulfhydration regulates Drp1 phosphorylation, GTPase activity, and inhibits its activity and translocation to mitochondria, thus attenuating mitochondrial over fission. In addition, Drp1 S-sulfhydration attenuates cardiomyocyte apoptosis by reducing 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 hydrogen sulfide on cardiac function[80]. S-sulfhydration is a novel Drp1 PTM potentially therapeutic for cardiovascular diseases.

Acetylation

Acetylation represents another major PTM in cells[81]. During the late 1990s, the first histone acetyltransferases (HATS) and histone deacetylases (HDACs) were cloned[82,83]. According to recent research, heart failure is associated with hyperacetylation of myocardial mitochondrial proteins[84,85], which may be due to an increase in acetyl coenzyme a utilization or a disruption of NAD+ homeostasis, which inhibits SIRT3 deacetylase activity[86]. A critical factor to be aware of 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), which leads to cardiomyocyte dysfunction and death. Excess lipid supply creates an intracellular environment that promotes Drp1 acetylation, which in turn leads to increased activity of Drp1 and mitochondrial translocation[88]. This suggests that Drp1 acetylation can cause cardiomyocyte death and dysfunction as a result of metabolic stress. Thus, Drp1 acetylation may represent one of the key PTMs that contribute 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 is implicated in the pathogenesis of a variety of diseases, including cancer, diabetes, neurodegenerative and cardiovascular diseases, among others[91-93]. Cardiac-specific OGT knockout mice show increased cardiac dysfunction after myocardial infarction, suggesting that O-GlcNAcylation has a crucial role in preserving cardiac function[94]. It has been demonstrated that 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 mitochondria[95]. Cardiomyocytes treated with high glucose induce O-GlcNAcylation at Drp1 threonine 585 (T585) and threonine 586 (T586), resulting in decreased Drp1 phosphorylation at Ser637. As a result, Drp1 activity and mitochondrial breakage were further increased, inhibiting OGA and impairing mitochondrial function in cardiomyocytes[95]. Alternatively, knockdown of OGT decreased Drp1 Ser637 phosphorylation levels and increased Drp1 translocation from the cytoplasm to the mitochondria in mice models of cerebral ischemia/reperfusion injury. Infarct volume and neurological function scores increased significantly after OGT knockdown, as well as levels of cleaved caspase-3 and neuronal apoptosis[96]. Accordingly, O-GlcNAcylation of Drp1 plays a different role under various pathological conditions, and may contribute to diabetes-induced mitochondrial dysfunction, such as diabetic cardiomyopathy. However, it remains to be determined how O-GlcNAcylation of Drp1 functions during myocardial ischemia

Ubiquitination

Ubiquitination is a key PTM that regulates biological functions through the

covalent-attachment of ubiquitin consisting of 76 amino acids to target proteins, changing their structure or forming a tag for proteasome degradation[97]. During ubiquitination, three types of enzymes are involved: ubiquitin activating enzymes (E1S), ubiquitin conjugating enzymes (E2S), and ubiquitin-protein ligases (E3S). As a starting point, ubiquitin is activated by the combination of E1S and adenosine triphosphate. Activated ubiquitin is then transferred to E2S, and this is followed by its covalent attachment 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 ubiquitinating Drp1, March5 participates in the regulation of mitochondrial morphology, leading to Drp1 degradation by the proteasome and reduced mitochondrial fission[101]. The ubiquitination of MARCH5-Drp1 can, however, also promote mitochondrial fission by facilitating the recruitment of Drp1 to specific fission sites[102]. Recent studies have demonstrated that Drp1 regulates MARCH5[103], completing the mechanism by which Drp1 is ubiquitinated. Parkin-Drp1 ubiquitination further promotes Drp1 degradation and reduces mitochondrial fission activity through a proteasome-dependent pathway[100]. This is consistent with the fact that a reduction in Drp1 degradation caused by Parkin knockouts or pathogenic mutations will result in excessive mitochondrial fission and, ultimately, the disease[35]. Currently, there is no indication that Drp1 ubiquitination contributes to cardiac diseases. It is necessary to determine whether it is associated with altered cardiac structure and function, and research in this area has great potential.

S-palmitoylation

In S-palmitoylation, palmitate is attached to cysteine residues of proteins via a reversible thioester bond by palmitoyl transferase of a zinc finger 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]. In this, Drp1 S-palmitoylation is a key mechanism for Drp1 translocation to mitochondria and the normal fission-fusion process. In addition to altering Drp1 activity, it also affects mitochondrial ATP production and switches the glycolytic glutamate (Glu) and γ-aminobutyric acid (GABA) cycles[106]. The results showed that there is a direct protein-protein interaction between Zdhhc13 and Drp1 in vivo and in vitro. In mice carrying the spontaneous stealth mutant Zdhhc13 gene (LUC), Drp1 S-palmitoylation is reduced, resulting in abnormal co-localization of Drp1 with mitochondria and altered mitochondrial morphology and distribution. Ultimately, this disrupts mitochondrial dynamics and leads to abnormal mitochondrial mitosis, resulting in cancer or cardiac arrhythmias[106]. There are currently only a few studies on Drp1 S-palmitoylation, however preliminary evidence suggests that it plays a key role. The altered status of Drp1 S-palmitoylation in different diseases and the regulatory mechanisms remain to be studied, particularly in the cardiac field, where altered Drp1 activity is associated with a series of important pathophysiological processes, making its S-palmitoylation of high value for research.

Targeting Drp1-modified pathway for treatment of heart diseases

It is feasible to prevent and treat cardiac dysfunction by targeting Drp1 modifications with drugs since Drp1-dependent mitochondrial division contributes significantly to cardiac disease development and progression and PTMs can dynamically regulate their activity[107]. Drp1 PTMs have been shown to be altered by a number of drugs. A small molecule inhibitor of Drp1 has been identified as mitochondrial division inhibitor 1 (mdivi-1). In diabetic cardiomyopathy, mdivi-1 treatment significantly reduced angiotensin II-induced Drp1 S616 phosphorylation and subsequently myocardial I/R injury[19,108], as well as increased ATP levels and mitochondrial complex (I, IV, and V) activity levels. Mdivi-1, however, may promote the accumulation of damaged mitochondria and impair cardiac function when used long-term in the treatment of myocardial hypertrophy and diabetic cardiomyopathy[109]. In addition, astragaloside IV derivative (LS-102) decreases phosphorylation of

Drp1Ser616 and increases phosphorylation of Drp1Ser637, thereby blocking I/R-induced mitochondrial division in order to protect cardiac function[110]. Emagliflzin prevents mitochondrial division by activating AMPK, inhibiting the phosphorylation of Drp1 S616 and increasing the phosphorylation of Drp1 S637; additionally, it inhibits mitochondrial reactive oxygen species (MtROS) production and subsequent oxidative stress to prevent the senescence of cardiac microvascular endothelial cells (CMEC). As a result, the barrier function of CMEC was preserved and structure and function of diabetic cardiomyopathy were improved[111]. Similar to Emagliflzin, Melatonin also blunts Drp1-dependent mitochondrial fission by activating AMPKα to downregulate Drp1 S616 phosphorylation and upregulate Drp1 S637 phosphorylation. By restoring the mitochondrial voltage-dependent anion channel (VDAC1), Melatonin interacts with hexokinase 2 (HK2), hindering MPTP opening and PINK1/Parkin activation, which ultimately prevents mitosis-mediated cell death and reduces I/R damage[112]. There are no studies that have shown that long-term melatonin use negatively affects Drp1. The same target is also activated by Ophiopogonin D (OP-D), which increases Drp1 activity by increasing Drp1 phosphorylation at Ser-616 and decreasing it phosphorylation at Ser-637, thereby reducing lipid accumulation and mitochondrial damage in diabetic mouse cardiomyocytes[113]. In addition to targeting Drp1 phosphorylation, zinc has been demonstrated to induce mitochondrial mitosis by increasing Drp1 SUMOylation, which leads to mitochondrial clearance and prevents ROS production during myocardial I/R injury, preserving cardiomyocyte function[70]. By overexpressing cystathionine gamma-lyase (CSE) or using exogenous sulfide, it is possible to increase hydrogen sulfide levels and prevent myocardial infarction and heart failure[78,79].

As a result of the discovery of many Drp1 PTMs and their loci, drugs targeting Drp1 PTMs have been developed, but most of them work on regulating the level 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 direction

Drp1 is a key protein for mitochondrial life activity, and heart failure models in cardiac-specific Drp1 knockout mice demonstrate the important role of Drp1 in maintaining normal cardiac function[114]. Regulation of Drp1 endogenous expression, Drp1 translocation to mitochondria, and Drp1-dependent mitosis are required to protect the heart from 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, as they affect cellular life activity by regulating protein levels and activity. This paper reviews the relationship between PTMs of the mitochondrial fission-dependent protein Drp1 and cardiac diseases. It is shown that dysregulation of numerous PTMs such as SUMOylation, phosphorylation, acetylation, O-GlcNAcylation, and S-sulfhydration of Drp1 can cause cardiac dysfunction. These PTMs promote or inhibit Drp1 activity, and their excessive activation or inhibition can lead to excessive mitochondrial fission or inability of damaged mitochondria to perform autophagy, which in turn leads to dysregulation of the dynamic balance of mitochondria and ultimately cell damage or even apoptosis. This is especially true in terms of mitochondria-dependent cells, such as cardiomyocytes and skeletal muscle cells. When mitochondrial dynamics is the therapeutic target under pathological conditions, it is critical to control the degree of mitochondrial fusion and fission, which should not be maintained at "normal" levels under all conditions. For instance, the physiological upregulation of mitochondrial fission in cardiac muscle under stress is requisite 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, as targeting mitochondrial fission is a double-edged sword. The dynamic balance of mitochondria assumes the most pivotal function, and how to maintain this balance is an important issue that needs to be addressed in the future.

Single PTMs are clearly essential in regulating protein structure-function relationships,

but until recently, different modifications have gradually been discovered to interact with each other through cooperation or competition. This crosstalk of PTMs has the potential to act 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 led to reciprocal regulation of phosphorylation at more than 400 sites of multiple proteins (280 of which had reduced phosphorylation levels[115], and functional crosstalk between O-GlcNAc or phosphorylation of Drp1 has now been identified. For example, high glucose treatment in cardiomyocytes induces O-GlcNacylation at Drp1 threonine 585 (T585) and threonine 586 (T586), which decreases phosphorylation at Drp1 Ser637, 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 S616 site, which promotes its mitochondrial translocation and GTPase activity[88]. This functional crosstalk between protein PTMs will be an important direction for future research and may open new site-viable options for the treatment of cardiovascular disease therapy.

In summary, we have conducted a comprehensive review of several specific Drp1 PTMs and demonstrated how these modifications modulate Drp1 to produce different outcomes. Continued research to identify potential targets could help design more effective therapeutic strategies for cardiac diseases and further exploration of various Drp1 modification-related enzymes and loci will help to better understand 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, as the modifications themselves and the crosstalk between them can alter their activities and thus affect the development of diseases. Based on the current situation, it is our belief that there remain several outstanding questions that require further attention and consideration: how to target and regulate the levels of PTMs of Drp1 without affecting other proteins? How to precisely alter the activity of Drp1 under different pathological conditions through PTMs? What are the specific mechanisms of functional crosstalk between different PTMs and what will be the results? The

resolution of these questions will contribute to the development of new therapeutic agents to reduce the morbidity and mortality of cardiovascular diseases.

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