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SERCA2a: a key protein in the Ca^{2+} cycle of the heart failure

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Abstract
Calcium ion (Ca^{2+}) cycle plays a crucial role in the contraction and relaxation of cardiomyocytes. The sarcoplasmic reticulum (SR) acts as an organelle for storing Ca^{2+}, which mediated the release and re-uptake of Ca^{2+} during contraction and relaxation. Disorders of SR function lead to the dysfunction of Ca^{2+} cycle and myocardial cell function. The sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) acts as a subtype of SERCA expressed in the heart, which mediates the contraction of cardiomyocytes and Ca^{2+} in the cytoplasm to re-enter into the SR. The rate of uptake of Ca^{2+} by the SR determines the rate of myocardial relaxation. The regulation of SERCA2a activity controls the contractility and relaxation of the heart, affecting cardiac function. The expression and activity of SERCA2a are reduced in failing hearts. Gene therapy by increasing the expression of SERCA2a in the heart has been proven effective. In addition, SERCA2a is regulated by a variety of factors, including transmembrane micropeptides, protein kinases, and post-translational modifications (PTMs). In this review, we discuss the regulatory factors of SERCA2a and provide new insights into future treatments and the direction of heart failure research. In addition, gene therapy for SERCA2a has recently emerged as therapeutic option and hence will be discussed in this review.

Keywords SERCA2a · Transmembrane micropeptides · PTM · Gene therapy

Introduction

With the aging population and the rising risk of cardiovascular disease, heart failure (HF) has become a global public health problem, which affects nearly 30 million people worldwide [1]. HF is the end stage of various heart diseases, which is called "the final battlefield" in the cardiovascular field. Drugs for clinical treatment of HF mainly include diuretics, angiotensin-converting enzyme inhibitors (ACE inhibitors) or angiotensin II receptor antagonists, and beta blockers [2]. However, heart failure still has high hospitalization and mortality rate.

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Ca^{2+} acts as a second messenger in the cellular process to mediate a variety of signaling pathways, and its role in the heart has been widely known [3–5]. In the heart, the sarcoplasmic reticulum (SR) is a Ca^{2+} reservoir and the intracellular L-type Ca^{2+} channel (LTCC), the ryanodine receptor 2 (RyR2), and the sarcoplasmic reticulum calcium pump (SERCA) maintain intracellular Ca^{2+} homeostasis, which converts electrical signals into cardiac contractility [6]. Depolarization of the cell membrane allows extracellular Ca^{2+} to enter the cytosol through the LTCC located in the T-tubule, and the gap between LTCC and SR is narrow, which provides conditions for the transient increase of intracellular Ca^{2+}. Extracellular Ca^{2+} triggers RyR2 on the SR membrane to release a large amount of Ca^{2+} from SR, and the cytosol Ca^{2+} concentration rises to a critical level, which triggers Ca^{2+} binding to troponin C and promotes coarse/thin filament slip. These series of reactions trigger a cardiac contraction effect. The diastolic effect of the heart is caused by SERCA2a (the major subtype of SERCA in the heart) and the Na/Ca^{2+} exchanger on the cell membrane that takes Ca^{2+} back into the SR (more than about 75% Ca^{2+}) and excreted from the cell [7] (Fig. 1). The activity and function of SERCA2a are impaired in HF. On the one hand, the decrease in SERCA2a activity reduces the Ca^{2+} content of SR, thereby reducing the amount of calcium ions released in the sarcoplasmic reticulum and

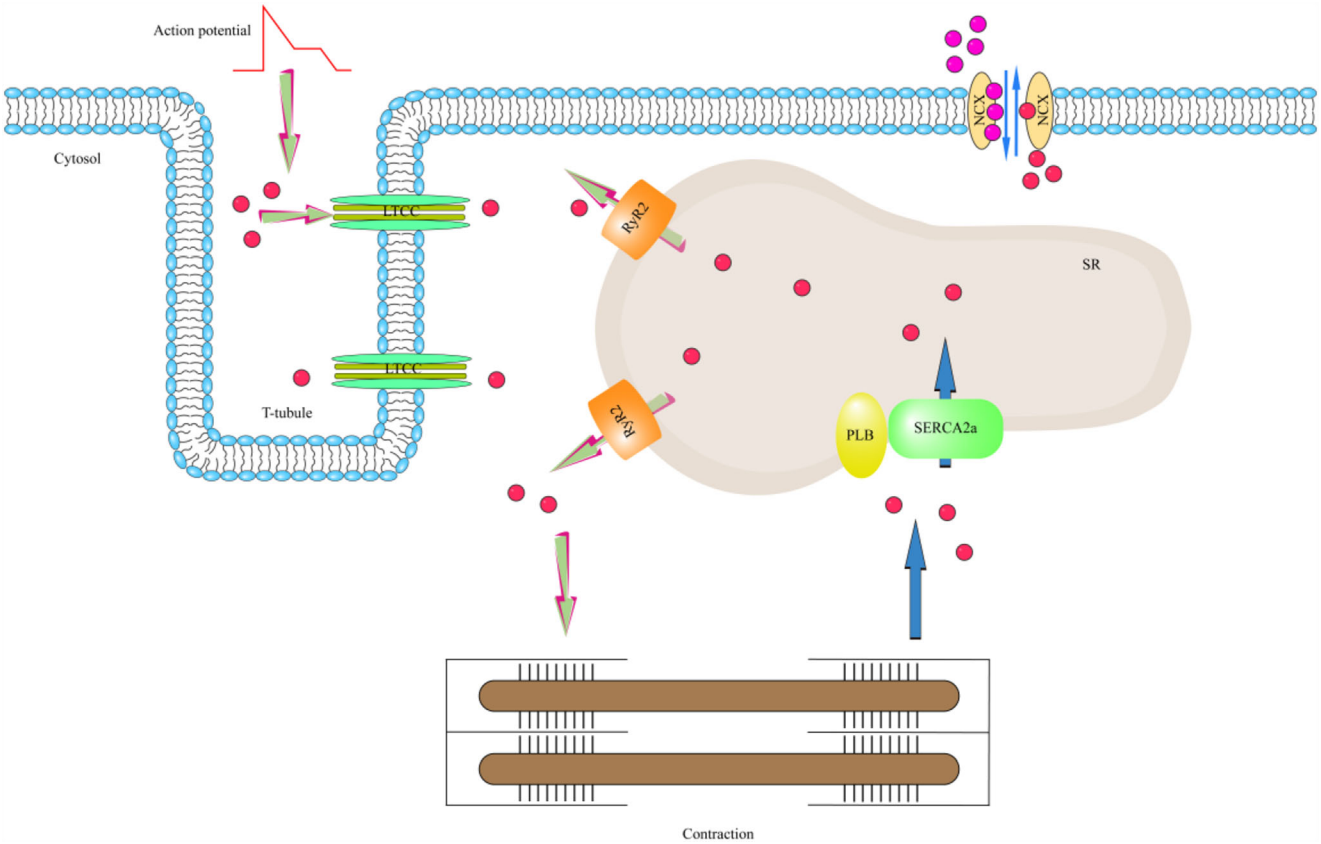

Fig. 1 Calcium cycle in cardiomyocytes. During systole, in the presence of membrane depolarization, calcium ions enter the cytosol through LTCCs, which triggers a large amount of Ca^{2+} in the SR to enter the cytoplasm through RyR2. After reaching the critical concentration, Ca^{2+} bind to troponin C and the myofilament slides. During diastole, a large amount of Ca^{2+} re-uptake into the SR through SERCA2a, and the remaining Ca^{2+} are excreted from the cells through NCX. Ca^{2+} are represented by red balls; Na^{+} are represented by purple balls. Two-color arrows indicate the contraction process and blue arrows indicate the diastolic process. LTCC, voltage-dependent L-type Ca^{2+} channel; RyR2, ryanodine receptor 2; SR, sarcoplasmic reticulum; SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; PLB, phospholamban; NCX, Na^{+}/Ca^{2+} exchanger

causing systolic dysfunction. On the other hand, loss of SERCA2a activity also reduces the amount and rate of calcium removal from the cytoplasm, which inhibits myocardial relaxation and diastolic dysfunction to some extent [8]. SERCA2a is regulated by a variety of transmembrane micropeptides. In addition to the previously studied phospholamban (PLB) and sarcolipin (SLN), some newly identified micropeptides such as dwarf open reading frame (DWORF) [9], another-regulin (ALN) [10], and myoregulin (MLN) [11] also regulate the activity of SERCA2a. With the development of proteomics, post-translational modification (PTM) has also been found to serve as a new target for the regulation of SERCA2a activity. For example, acetylation [12], SUMOylation [13], and nitrification [14] and their corresponding PTMs will be discussed.

Specifically, in this review, we will describe SERCA2a and its related regulatory factors, with emphasis on the therapeutic potential of PTMs. In addition, we will also cover the recent gene therapy of SERCA2a. Recent studies have used SERCA2a as a strategic prospect for the treatment of heart failure.

SERCA2a is a Ca^{2+} refluxor

The mammalian SERCA family has several subtypes encoded by SERCA1, SERCA2, and SERCA3, wherein SERCA2a (encoded by the ATP2A2 gene) and SERCA1a (encoded by the ATP2A1 gene) have strong similarities and 84% protein sequence identity [15]. Although the expression position is not consistent, SERCA2a is mainly expressed in cardiomyocytes and type I skeletal muscle, while SERCA1a is mainly expressed in type II skeletal muscle, but the consistency of structure and sequence indicates that the Ca^{2+} transport mechanism may be highly conserved [16]. SERCA2a is a key protein in the calcium cycle of cardiomyocytes, which is used to re-uptake Ca^{2+} in the cytoplasm into the sarcoplasmic reticulum to relax the cardiomyocytes, thereby maintaining Ca^{2+} homeostasis in cells. After the cardiomyocytes complete a contraction action, SERCA2a takes up Ca^{2+} and returns to SR [17]. This process involves the mutual transformation of the two structures of the SERCA2a protein: E1 and E2. The E1 structure of SERCA2a has a higher affinity for Ca^{2+}, and

the binding site is exposed to SR. However, the E2 structure has a lower affinity for Ca^{2+}, which is located on the lumen of the SR. After relaxation of cardiomyocytes, Ca^{2+} is released from troponin into the cytosol and binds to E1 (the binding site is at the aspartic acid residue), after which phosphorylation of E1 occurs with the participation of ATP (Ca^{2+}-E1-P), which then causes a conformational change from E1 to E2 (CA^{2+}-E2-P), and the E2 structure becomes an ADP-insensitive intermediate [18, 19]. This process results in the localization of the Ca^{2+} binding site in the SR. Due to the low affinity of E2 for Ca^{2+}, Ca^{2+} is released into the SR after uncoupling with E2, accompanied by E2 converted to E1 and re-entering the next cycle (Fig. 2).

SERCA2a and its regulator—transmembrane micropeptide

PLB and SLN have been shown to bind to the cytoplasmic/transmembrane domains of SERCA2a, which inhibits the affinity of SERCA2a for Ca^{2+} [20]. As a major regulator of SERCA2a activity, PLB is the only regulatory protein of SERCA2a directly involved in the development of cardiac disease, including HF.

PLB

PLB is a 52–amino acid single transmembrane protein expressed in the SR, which exists as a pentamer and regulates the activity of SERCA2a [21]. It was first discovered by James and his colleagues that PLB interacts with SERCA2a and that non-phosphorylated PLB inhibits SERCA2a activity [22], which can be phosphorylated by cAMP-dependent protein kinase (PKA) and calcium/calmodulin-dependent protein kinase II (CaMKII). SERCA2a undergoes conformational change (become a calcium binding state) to relieve the inhibition of PLB [23]. Subsequent research focused on the location of SERCA2a in response to PLB inhibition. SERCA2 has two key domains: one is the region between amino acids 336 and 412 in the phosphorylation domain, which responds to the action of PLB; the other is the amino acids 467 and 762 in the nucleotide-binding domain, which mainly determines the strength of SERCA2 for Ca^{2+} affinity. These two domains collectively respond to phosphorylated PLB to shift the conformation of SERCA2 from a non-calcium-bound state to a calcium-bound state, thereby enhancing the Ca^{2+} affinity of SERCA2. Toyofuku et al. further revealed that the cytoplasmic 1A domain of PLB plays a key role in the function of SERCA2a through mutations in PLB residues [24].

The regulation of SERCA2a activity by PLB involves two steps: first, the dissociation of PLB from a pentamer to a monomer, which is a reversible process. The monomeric PLB then combines with SERCA2a to form a PLB-SERCA2a complex, thereby exerting an inhibitory effect. The increase in SERCA2a activity is regulated by phosphorylation of PLB and Ca^{2+} [25] (Fig. 3). On the one hand, PLB phosphorylation dissociates from the PLB-SERCA2a complex, which abolishes the inhibitory effect of PLB on SERCA2a. In addition, the increase of cytosolic Ca^{2+} concentration directly regulates the binding ability of SERCA2a to Ca^{2+}, which enhances the capacity of sarcoplasmic reticulum to regress Ca^{2+} and induce myocardial cell relaxation.

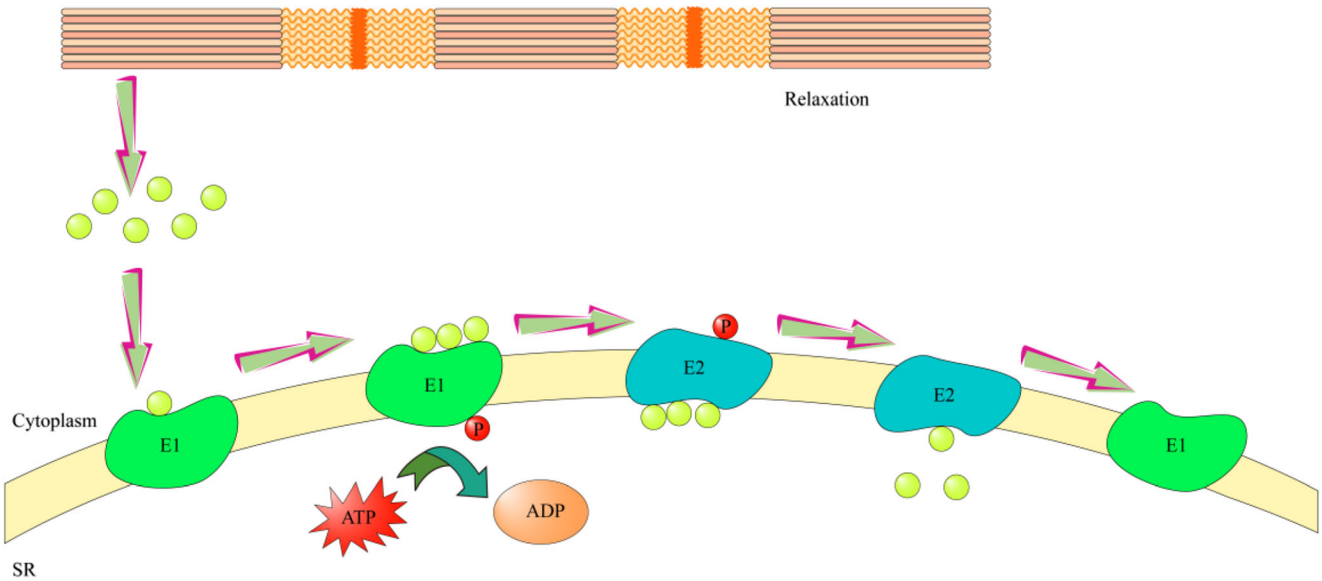

Fig. 2 The mechanism of action of SERCA2a. After relaxation of the heart, Ca^{2+} bind to SERCA2a in the E1 state, and with the participation of ATP, the conformational change of E1 is converted to E2. Ca^{2+} are transported into SR, and intracellular Ca^{2+} concentration restores resting level. Ca^{2+} are represented by green balls; Pi are represented by red balls. SR, sarcoplasmic reticulum; E1, E1 structure of SERCA2a; E2, E2 structure of SERCA2a

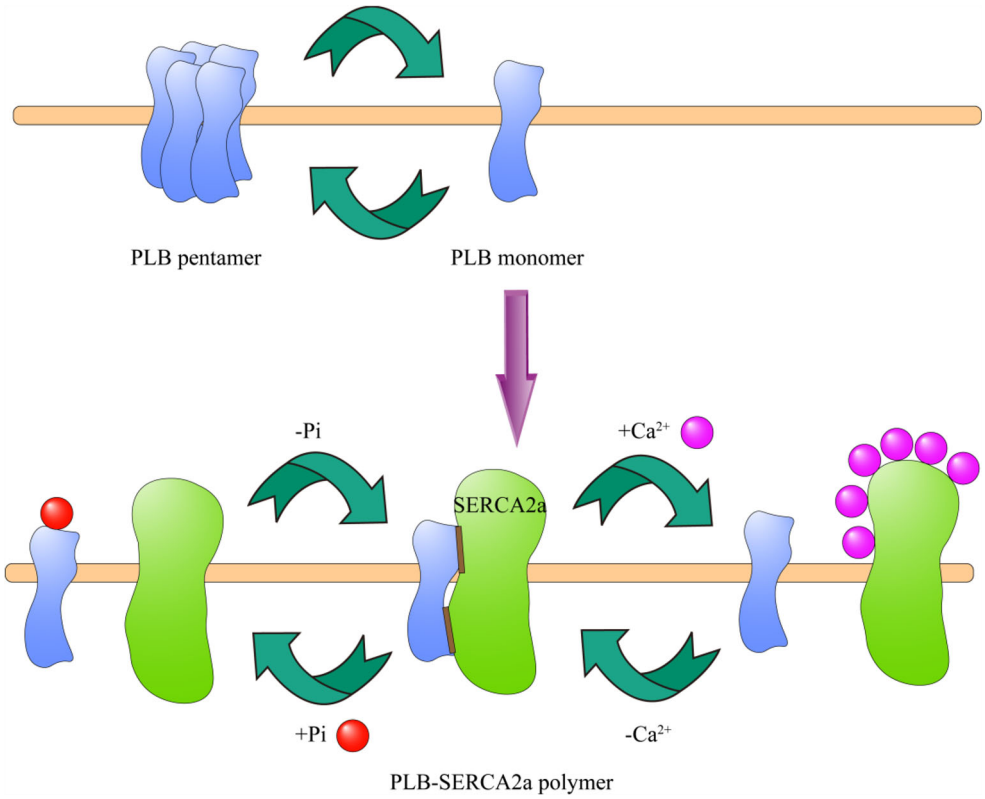

Fig. 3 Interaction mechanism of PLB-SERCA2a. The PLB pentamer can be depolymerized into a monomer, which is a reversible process. PLB monomer can inhibit the binding of SERCA2a; there are two cases that can inhibit the inhibition of SERCA2a by PLB: the first case is phosphorylation of PLB and the second case is calcium with increasing Ca^{2+} concentration. Ca^{2+} binding to SERCA2a will relieve PLB inhibition. SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; PLB, phospholamban

In recent years, a lot of research has been done on PLB and SERCA2a. Mazzocchi et al. [26] used PLB ablation to improve Ca^{2+} uptake by SERCA2a. Interestingly, it prevented arrhythmias caused by Ca^{2+} leakage triggered by excessive phosphorylation of RyR2. In addition, ablation of PLB has a cardioprotective effect on heart failure mice and reduces mortality in heart failure mice [27]. Another study found that PLB-deficient mice prevented arrhythmia during ischemia-reperfusion. It is worth noting that ablation of PLB significantly increased the infarct size of the heart, suggesting that ablation of PLB exacerbates ischemia-reperfusion injury [28]. The inconsistency between the tendency of reduced arrhythmia and the further deterioration of cardiac damage caused by ischemia-reperfusion justifies the involvement of many different regulatory mechanisms. In addition, PLB is related to genetic predispositions. In a study conducted by Schmitt and colleagues [29], they found that the phosphorylation of PLB was impaired and the Ca^{2+} binding ability of SERCA2a was weakened after the missense mutation of arginine at position 9 of PLB amino acid residue was changed to cysteine. Studies show abnormal Ca^{2+} treatment and impaired cardiac function. With the development of proteomics technology, the cognitive molecules of PLB are more widely recognized. Protein phosphatase 1 (PP1) is an endogenous dephosphorylation enzyme of PLB, and the newly identified PP1 regulatory subunit (PPP1R3A) binds to PLB. PPP1R3A knockout mice generated by deletion of exon fragments by Alsina et al. found that PP1-mediated targeting of RyR2 and PLB dephosphorylation was impaired, and excessive SERCA2a and RyR2 activity was highly likely to be triggered by atrial fibrillation [30]. In recent years, the emergence of near-infrared fluorescence nanoprobe has also provided us with a more efficient and convenient way to identify transmembrane micropeptides. Zhan et al. [31] designed specific probes for PLB and identified a range of properties, including sensitivity, specificity, and effects on PLB, which contribute to early diagnosis of heart failure. The emergence of new technologies will guide us in the far-reaching function of PLB.

SLN

SLN is another transmembrane micropeptide present on SR, consisting of 31 amino acids, which is mainly expressed in the atria. SLN shares the same protein family as PLB. The SLN contains a short dynamic helix and a rigid helix, and two short unstructured terminuses: one at the N-terminal 1–6 residues and the other at the C-terminal 27–31 residues [32]. SLN was found later than PLB, which was first identified on the skeletal muscle SR of rabbits. Therefore, earlier studies have tended to study the role of SLN in SERCA1 [33, 34]. Similar to the effect of PLB on SERCA2a, SLN can inhibit SERCA1a [35]. Initially, Asahi and colleagues [36] used mutations in SLN and PLB and co-immunoprecipitation of SERCA to show that SLN can bind to PLB, which in turn forms a

SLN-PLB-SERCA complex that has a higher inhibitory effect on SERCA. Moreover, the SLN-PLB complex prevents the polymerization of PLB (cannot be converted from a monomeric form to a pentamer), which has a sustained inhibitory effect on SERCA. Paradoxically, SLN still has an inhibitory effect on SERCA in tissues (atrial, slow-shrinking skeletal muscle) with low PLB expression or no expression. Michio et al. [37] found in mice with cardiac-specific overexpression of SLN that SERCA2a has reduced affinity for Ca^{2+}. In addition, adenovirus-mediated SLN overexpression prolonged 50% relaxation and Ca^{2+} decay time with impaired cardiac function [38]. Ablation of SLN does not alter PLB protein expression and phosphorylation, and enhances SERCA affinity for Ca^{2+} and the maximum rate of Ca^{2+} uptake [39, 40]. These studies show that SLN does not rely on PLB to function but directly binds to SERCA to exert inhibition. Interestingly, SLN has a different effect on SERCA than PLB. Under high concentration of Ca^{2+}, SLN still plays a role in the inhibition of SERCA [41], which is contrary to the inhibition of SERCA by PLB at high concentration of Ca^{2+}. SLN mainly reduces the maximal rate of Ca^{2+} uptake by interacting with SERCA, which is different from the regulation mechanism of PLB on ATPase activity of SERCA. The regulation of SLN is not affected by Ca^{2+} concentration [42]. In addition, SLN is also regulated by phosphorylation, and CaMKII phosphorylates the N-terminal threonine-5 residue of SLN [43]. Serine/threonine phosphokinase 16 also exerts phosphorylation of SLN, which in turn significantly reduces the inhibitory effect of SLN on SERCA [44]. In conclusion, SLN is an important regulator of myocardial SERCA, and its physiological function and structure require further research. Specific drugs for SLN have not yet been discovered, but various studies have demonstrated that it is a strategic approach to improve the Ca^{2+} cycle disorder in failing hearts by regulating SLN. This requires a combination of basic experiments and clinical validation.

Potential micropeptides of SERCA2a

In recent years, some newly discovered transmembrane micropeptides have been identified, which play a novel role in the regulation of SERCA2a.

DWORF removes the inhibitors on SERCA2a

A 34–amino acid peptide was identified: DWORF, which was previously considered a non-coding RNA. DWORF can eliminate the inhibition of SERCA2a by replacing PLB, SLN, thereby enhancing the activity of SERCA2a. It was confirmed in the study of mouse cardiomyocytes and type I skeletal muscle that DWORF significantly affects intracellular Ca^{2+} treatment and enhances calcium pump activity [9]. DWORF has a higher affinity for SERCA than PLB, and overexpression of DWORF significantly improved cardiac function in dilated heart disease mice and improved dysregulated Ca^{2+} circulation [45]. These findings give us a preliminary understanding of DWORF. On the one hand, DWORF can be used as a biomarker in heart failure. The decrease in its level means that the contractility of the myocardium is affected. In addition, DWORF can be used as a target for new drugs. It is a strategic approach to increase the activity of SERCA2a by increasing the amount of DWORF in the heart, which will break through the limitations of modern positive inotropic drugs.

MLN/ELN/ALN: additional inhibitor of SERCA2a

Myoregulin (MLN) is a newly discovered functional micropeptide encoded by skeletal muscle–specific RNA, a micropeptide with 46 amino acids. MLN and PLB have similar effects on SERCA, and MLN is widely expressed in skeletal muscle as a suppressor of SERCA1. Deletion of MLN in mice revealed an increase in SERCA activity and improved Ca^{2+} handling [11]. In addition, endoregulin (ELN) and ALN are newly identified two transmembrane micropeptides whose N-terminal domain in the cytosol contains conserved sequences of serine and threonine residues. ELN mainly inhibits SERCA3 present in endothelial cells and epithelial cells. Notably, the N-terminal domain of ALN contains a motif that can be phosphorylated by PKA, which is similar to the phosphorylation motif of PLN. It is suggested that the inhibition of SERCA2b by ALN may be related to the inhibition of SERCA2a by PLN through the same conserved mechanism [10]. Interestingly, the C-terminal transmembrane helix of ELN, ALN, and MLN is similar to PLB and SLN, suggesting that these new transmembrane micropeptides are a conserved mechanism for Ca^{2+} regulation. However, there are few studies on the role of ELN, ALN, and MLN in the myocardium. Currently, enough evidence is unavailable concerning whether these new micropeptides have similar effects on SERCA2a like PLB and SLN. Whether ELN, ALN, and MLN have the same effect on SERCA2a and the combination of PLN or SLN is worthy of further study.

Multiple PTMs regulate the activity of SERCA2a

PTM refers to the covalent attachment of chemical small molecule groups to the amino acid side chain of a protein, significantly increasing the complexity and diversity of the protein [46]. More than 400 PTMs have been identified, PTM can occur in almost all proteins, and multiple PTMs can occur in the same protein [47]. More importantly, the PTM of the protein significantly changes the physicochemical properties and conformation of the protein, thereby directly changing the binding ability and function of the protein. Therefore, even

if the expression level of the protein does not change, the state of the post-translational modification changes the function of the protein significantly [48]. A number of studies have demonstrated that SERCA2a is capable of PTM and different types of PTM occur at different sites, which affects the activity and function of SERCA2a.

SUMOylation

The small ubiquitin-like modifier (SUMO) is an important member of the ubiquitin-like protein family, and its molecular structure is similar to that of ubiquitin, but the biological significance of these two types of protein modification is different. Ubiquitination mediates protein degradation, while the SUMO family is widely involved in cellular activities such as protein structural stability, nuclear translocation [49], and regulation of transcriptional activity [50]. The SUMO family mainly includes SUMO1, SUMO2, and SUMO3. Since SUMO2 and SUMO3 have a high degree of sequence identity, they are called SUMO2/3 [51], SUMO1 is mainly involved in delayed response, and SUMO2/3 is involved in acute stress response. SUMO was originally an immature precursor, which required the splicing of SUMO proteases (SENPs) to become activated forms. Activation of splicing of SUMO molecules is terminated by Gly residues [52]. SUMO modification involves a cascade of related enzymes: First, mature SUMO was first activated by SUMO-activating enzyme (E1), and ATP participated in this process. E1 is a heterodimeric protein complex composed of Uba2 and Aosl. The cysteine residues on SUMO and Uba2 were bound by thioester bonds with the participation of SUMO adenosine intermediates. Uba2 is only known as SUMO-binding enzyme (E2). The binding of SUMO to the substrate protein is accomplished by the action of SUMO ligase (E3). SUMO was linked to the substrate protein lysine side chain to form an isopeptide bond and exert PTM action [53]. SUMO modification is a reversible dynamic process. Under the enzymatic cleavage of SENPs, SUMO molecules dissociate from the substrate and re-enter the SUMO cycle.

In 2011, Kho et al. [54] found that the expression of SUMO1 and the SUMOylation of SERCA2a were significantly decreased in patients and mice subjected to heart failure. They used small hairpin RNA (shRNA) to downregulate SUMO1, which accelerated the deterioration of cardiac function and demonstrated that SUMOylation is essential for the activity and stability of SERCA2a. Overexpression of SUMO1 significantly improved cardiac function, including improvement of Ca^{2+} cycle in cardiomyocytes, and functional verification of SUMOylation of SERCA2a, followed by regulation of SUMOylation of SERCA2a as a research target for heart failure treatment. Kho [55] further characterized the small molecule N106 and found that N106 increased the SUMOylation level of SERCA2a, and a series of studies on this small molecule compound found that N106 indirectly triggered the endogenous SUMOylation of SERCA2a by directly activating E1. Treatment with N106 improves the contractility of cardiomyocytes and improves cardiac function in heart failure mice. This further demonstrates that SUMOylation by increased SERCA2a is a promising treatment, which also provides more new indicators for drug development. It is worth noting that the level of SUMO1 in the heart increased slightly during hypertrophy, but the level of heart failure decreased sharply [56]. The staged suggestion of SUMO1 levels has a more beneficial effect on gene therapy with SUMO1 in the compensatory phase. In addition, gene therapy of SUMO1 [57] has also been shown to be effective in a porcine model of ischemic heart, which is characterized by improved cardiac function (including ejection fraction, maximum rate of increase in pressure) and contractile relaxation effect of cardiomyocytes. Oh et al. [58] observed a strong correlation between miR-146a and SUMO1 in the heart of depleted mice and humans, and further studies of both showed elevated levels of miR-146a in the failing heart, which reduced the expression of SUMO1 and negatively regulated the SUMO1-SERCA2a axis. Additionally, a research by Du and co-researchers [59] used the cardiac ischemia-reperfusion mouse model to find the SUMOylation of SERCA2a and the decrease of SUMO1 in the heart. Further studies found that the SUMOylation receptor sites of mouse SERCA2a are lysine 585, 480, and 571. Treatment with luteolin improved heart function in heart failure mice and reduced infarct size in the heart, and demonstrated that luteolin regulates SERCA2a through SUML 585 to reduce myocardial ischemia-reperfusion injury. These studies suggest that improving cardiac function in heart failure by mediating SUMOylation of SERCA2a should be one of the notable targets in the development of new drugs.

Acetylation

The acetylation modification is the transfer of the acetyl group of the donor to the amino acid residue of the protein of interest by an enzymatic or spontaneous reaction, which modulates the function of the target protein. Protein acetylation is another PTM that regulates protein activity and function [60]. With the development of gene manipulation and proteomics, various functions following protein acetylation have attracted more attention. Acetylation is involved in transcriptional regulation, metabolic regulation, and signaling pathway regulation, and plays an important role in controlling protein conformation, activity, and stability [61]. For acetylation modification, current research hotspots focus on acetylation modification on histones, which is crucial in the process of apparent regulation [62]. However, the broader function of acetylation

modification is not limited to the nucleus, and the acetylation modification is also extensive in a large number of proteins in the cytoplasm and other sub-cells, called non-histone acetylation modification. Acetylation modification is mainly divided into two categories: one is acetylation at the amino acid residue at the end of the receptor protein and the other is acetylation at the lysine in the receptor protein chain [63]. The former is a covalent modification that occurs mostly in nascent proteins of eukaryotes and is important for the maturation and cellular localization of nascent proteins, and is responsible for N-acetyltransferases (NATs), which is a reversible acetylation modification. The acetyl-CoA acetyl group is mainly transferred to the ε-amino side chain of lysine by lysine acetyltransferase (KATs) to exert a regulatory effect and can be reversed by lysine deacetylase (KDACs). The reversible changes in protein or histone acetylation regulated by KATs and KDACs are associated with many common diseases, including heart disease [64], diabetes [65], neurodegenerative diseases [66], and cancer [67], as well as some rare diseases such as mitochondrial diseases [68].

In a recent study from humans, pigs, and mice [12], the researchers found a significant increase in the level of acetylation of SERCA2a in failing hearts, a phenomenon attributed to a decrease in the level of SIRT1 (a class III histone deacetylase). The acetylation of SERCA2a had the opposite effect as SUMOylation, and the increase in acetylation of SERCA2a significantly reduced the activity of SERCA2a, which caused a decrease in the uptake of Ca^{2+} and impaired cardiac function of SERCA2a. The acetylation level of lysine 492 (K492) was found to be responsible for SERCA2a dysfunction by measuring the acetylation site. The histone acetyltransferase p300–mediated K492 acetylation reduced the ATP binding ability of SERCA2a. This study points us to new research ideas, on the one hand, blocking the acetylation of SERCA2a mediated by p300, thereby reducing the level of acetylation of SERCA2a, which indirectly improves the function of damaged SERCA2a in failing hearts. On the other hand, the acetylation level of SERCA2a is lowered by increasing the level of SIRT1, which has been shown to have high acetylation of SERCA2a. It is a strategic approach to develop a new drug or small molecule that mediates the reduction of SECCA2a acetylation levels from the source and on the way of SERCA2a [69]. It is worth noting that acetylation mediates a variety of physiological effects and widely expressed in vivo, and the side effects produced after activation of SIRT1 will need to be considered. In addition, drug development by combining SUMOylation and acetylation of SERCA2a may result in better balance and fewer side effects.

Phosphorylation and O-GlcNAc modification

In addition to SUMO and acetylation, SERCA2a is also affected by other PTMs. Phosphorylation of proteins is the most widely studied and plays an important role in protein PTM, which is involved in the regulation of various important molecular activities and functions. Striated muscle–specific protein kinase (SPEG), as a member of the myosin light chain kinase (MLCK) subgroup of the CaMK Ser/Thr protein kinase family, is closely related to phosphorylation of SERCA2a. Quick et al. [70] used proteomics to discover that SERCA2a interacts with SPEG, and they further used cell lines and primary cardiomyocytes of neonatal rats to demonstrate that the second kinase domain of SPEG acts on SERCA2a, which relies on SPEG to directly phosphorylated the Thr484 site of SERCA2a, thereby enhancing the oligomerization of SERCA2a and increased the Ca^{2+} transport capacity of SERCA2a. Further study [71] of SPEG-SERCA2a through used SPEG cardiac-specific knockout mice revealed that phosphorylation of the Thr484 site of SERCA2a and the level of oligomerization of SERCA2a were significantly reduced in the hearts of mice deficient in SPEG, which inhibited the activity of SERCA2a and impaired Ca^{2+} uptake in the sarcoplasmic reticulum of the cardiomyocytes, which was subsequently accompanied by maladaptive cardiac function in mice leading to the death of these mice. In addition, Li and subordinates [14] found that iron supplementation stimulated cardiomyocyte hypertrophy and led to increased formation of cardiac protein carbonyl tyrosine, which resulted in abnormal myocardial calcium homeostasis in diabetic rats. Further studies in vitro have revealed that tyrosine nitration of SERCA2a played an important role in the activity of SERCA2a, which reduced the ability of SERCA2a to modulate Ca^{2+} and damaged SERCA2a activity. Intracellular protein translation was followed by N-acetylglucosamine (O-GlcNAc) modification, catalyzed by O-GlcNAC glycosyltransferase, which is involved in the regulation of many important biological processes in cells, and application in human diseases and treatment [72]. O-GlcNAc has been shown to modify SERCA2 protein levels and prevent Ca^{2+} treatment of SERCA2a. In addition to modifying SERCA2a protein levels, O-GlcNAc can also modify SERCA2a activity by regulating phosphorylation of PLB [73]. In conclusion, O-GlcNAc regulates the activity of SERCA2a either directly or indirectly. In addition, NO is a physiological regulator that stimulates SERCA2a to accelerate the reduction of intracellular Ca^{2+} concentration, which relaxes the heart muscle, skeletal muscle, and smooth muscle. NO-derived intermediates can also play a role in protein modification. Lancel et al. [74] clarified the molecular mechanism by which nitroxyl partially exerts positive inotropic and relaxing effects in the myocardium by enhancing the activity of SERCA2a. They found that nitroxyl passes S-glutathionylation at cysteine 674 and increased the activity of SERCA2a, which enhanced the contractile relaxation effect of cardiomyocytes.

SERCA2a is affected by a variety of PTMs, and it may be a promising direction to mediate PTM in clinical or

Table 1 Randomized clinical trial results of SERCA2a gene transfer in HF

Trial	Numbers	Subjects	Efficacy	Safety	Status	Reference
CUPID: a phase 1/2 trial	9	Advanced HF (New York Heart Association [NYHA] class III/IV, ejection fraction [EF] < or = 30%), 6–12-month follow-up	Multiple parameters were improved, including symptomatic (NYHA and Minnesota Living with Heart Failure Questionnaire, 5 patients), functional (6-min walk test and VO2 max, 4 patients), biomarker (NT-proBNP, 2 patients), and LV function/remodeling (EF and end-systolic volume, 5 patients)	No difference vs. placebo	Precursor to CUPID 2	[79]
CUPID: a phase 1/2 trial	39	Advanced HF (NYHA class III/IV, ejection fraction [EF] < or = 30%), 6–12-month follow-up	The frequency of cardiovascular events decreased, and the mean time to cardiovascular hospitalization was significantly reduced within 12 months of high-dose therapy compared with placebo	No difference vs. placebo	Precursor to CUPID 2b	[80, 83]
CUPID: a phase 2b trial	250	Enrolled patients will be at high risk for recurrent HF hospitalizations by virtue of having elevated N-terminal pro-B-type natriuretic peptide/BNP (> 1200 pg/ml) and/or recent HF hospitalization, 6–12-month follow-up	AAV1/SERCA2a at the dose tested did not improve the clinical course of patients with heart failure and reduced ejection fraction, and did not improve time to recurrent events compared with placebo	No difference vs. placebo	No further HF trials planned	[81, 84]
AGENT-HF trial	9	NYHA class III–IV ischemic or non-ischemic heart failure and left ventricular ejection fraction ≤ 35%, 6-month follow-up	AGENT-HF failed to demonstrate any improvement in ventricular remodeling in response to AAV1/SERCA2a at the dose studied	No difference vs. placebo	Suspend	[85]

experimental settings to improve reduced activity in heart failure. It is worth noting that the effects of PTM changes on other functional proteins have not been revealed, which requires us to study more in depth.

Gene therapy approaches to increase SERCA2a pump activity—stimulating the calcium pumps

With the development of molecular biology, transgenic technology, and vector technology, gene therapy has gradually become a new breakthrough in heart failure treatment. Decreased expression and activity of SERCA2a in heart failure are key links in the Ca^{2+} cycle. Therefore, overexpression of SERCA2a by gene transduction technology has become a key point in gene therapy for heart failure. Studies of genetic alteration models have identified the functional role of the SERCA pump in Ca^{2+} handling and cardiac physiology. A series of cell models, rodent models, and large animal models have shown that the introduction of SERCA2a gene into viral cardiomyocytes with viral vectors can improve systolic and diastolic effects [75–77]. After occluding the proximal left anterior descending balloon of the pig and then reperfusion for 1 month, adeno-associated vector type 1 (AAV1)–mediated transgenic therapy of SERCA2a significantly improved cardiac function and prevented expansion of left ventricular volume [57]. In 2007, the first clinical trial of heart failure to SERCA2a gene therapy began in the USA [78]. This study used AAV1 with SERCA2a to treat patients with advanced heart failure by direct intracoronary injection, which was used to determine the effectiveness and safety of different doses of AAV/SERCA2a. The study found that in the high-dose AAV1-SERCA2a group, the improvement in cardiac function and the 6-min walking distance were significantly better than in the placebo group [79]. After 3 years of follow-up, the major adverse cardiovascular events (including recurrent myocardial infarction, worsening HF, and rehospitalization) were significantly lower than in the placebo group [80]. Based on previous findings, a larger sample size study was conducted in 2012, which included 250 patients with advanced heart failure. Surprisingly, the AAV1-SERCA2a group showed a major endpoint compared with the placebo group. No significant gains were found in the secondary endpoints [81]. After that, RT-PCR analysis of tissue samples from patients undergoing heart transplantation or left ventricular assist device implantation and death found that the amount of vector DNA in the cardiomyocytes of patients in the second clinical trial was much lower than that in the first phase. Despite the small number of tissue samples, this suggested that the failure of the second clinical trial may be due to the low transfection rate of SERCA2a. Although the large sample clinical trial failed, the trial confirmed that the SERCA2a gene is safe for heart failure. Gene therapy remains the most promising treatment for diseases. Problems encountered in SERCA2a gene therapy are expected to be resolved in the future. At present, it has been found that the purpose of improving the efficiency of intracoronary injection can be improved by establishing a closed loop system for recycling [82]. In addition, the use of immunosuppressive drugs or plasmapheresis methods may reduce the effects of AAV neutralizing antibodies, or the development of vector engineering and recombinant technology to improve viral vector safety and transfection efficiency. With the development of gene transduction technology and novel gene therapy vector system, gene therapy will make breakthrough progress in the near future.

Perspectives

A variety of evidences demonstrate the importance of SERCA2a in maintaining normal heart function (Table 1). Several studies have shown that gene therapy methods for SERCA2a expression are effective in clinical trials [79, 83]. However, subsequent large-scale clinical trials have not been validated [84, 85]. Although no evidence of improved prognosis was found at the AAV1/SERCA2a dose studied, these trials spurred further studies using gene therapy to treat HF. A variety of means for regulating SERCA2a-associated transmembrane micropeptides are also being investigated. It is worth considering that the regulation of SERCA2a by myriads of emerging PTMs cannot be ignored. SERCA2a-mediated gene therapy increases SERCA2a expression but does not alter SERCA2a PTM. The discovery of small molecules, such as N106, improves the activity of SERCA2a by increasing the level of SUMOylation of SERCA2a [55]. Gene therapy for SUMO1 has also been shown to be effective in the heart of pigs [57]. In addition, elevated acetylation of SERCA2a in newly discovered heart failure patients may also be a new strategy by reducing the level of acetylation of SERCA2a in failing hearts [12]. Drug discovery targeting the PTM of SERCA2a will be an impeccable therapeutic option for people with heart failure in the near future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Abbreviations AAV1, adeno-associated vector type 1; ALN, another-regulin; Ca^{2+}, calcium ion; CaMKII, calcium/calmodulin-dependent protein kinase II; DWORF, dwarf open reading frame; ELN, endoregulin; HF, heart failure; KATs, lysine acetyltransferases; KDACs, lysine deacetylase; LTCC, L-type Ca^{2+} channel; MLN, myoregulin; NATs, N-acetyltransferases; PLB, phospholamban; PKA, cAMP-dependent protein kinase; PP1, protein phosphatase 1; PTM, post-translational modification; RyR2, ryanodine receptor 2; SERCA2a, sarco/endoplasmic reticulum Ca^{2+} ATPase 2a; SLN, sarcolipin; SPEG, striated muscle–specific protein kinase; SR, sarcoplasmic reticulum; SUMO, small ubiquitin-like modifier

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