SPEG, an Indispensable Kinase of SERCA2a for Calcium Homeostasis

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Heart failure is one of the major causes of death worldwide. Despite the development of several treatments for heart failure, such as β-blocker, angiotensin-converting enzyme inhibitor, and mineralocorticoid receptor antagonist, most severe heart failures irreversibly progress and cannot be cured without heart transplantation. In the excitation-contraction coupling in cardiomyocytes, Ca\(^{2+}\) reuptake into the sarcoplasmic reticulum (SR) through SERCA2a (SR Ca\(^{2+}\) ATPase 2a) is a key process in the relaxation of cardiomyocytes and in the proper storage of SR Ca\(^{2+}\) content for the next contraction.\(^1\) SERCA2a is downregulated in heart failure, and Ca\(^{2+}\) reuptake to SR is reduced in diseased cardiomyocytes. As a result, excitation-contraction coupling is impaired in systolic and diastolic phases, causing a vicious spiral of SERCA2a decrement and heart failure. Hence, correcting impaired intracellular Ca\(^{2+}\) homeostasis could be a therapeutic target. In heart failure animal models, overexpression of SERCA2a improved cardiac function,\(^2\) and gene therapy delivering the SERCA2a gene for heart failure treatment is expected to be highly successful in humans. Clinical studies, CUPID 1\(^{1}\) and CUPID 2 (Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease),\(^3\) have already been conducted using adeno-associated virus carrying a SERCA2a gene, but the improvement in prognosis has not been clearly shown. This inconsistency in results may be attributable to the difference in study designs and factors such as patient characteristics, virus dosage, and the gene delivery system. Thus, these factors should be adjusted in future clinical trials. Moreover, a drug that could directly affect the SERCA2a function is also expected to be developed. SERCA2a activity is finely regulated by several mechanisms, and the modulation of SERCA2a activity is critical for efficient Ca\(^{2+}\) reuptake into SR. PLN (phospholamban) is highly expressed in cardiomyocytes and reversibly inhibits SERCA2a activity.

Adeno-associated virus–mediated Pln knockdown rescued heart failure in an animal model.\(^4\) The activity and stability of SERCA2a is also regulated by post-translational modification, including the SUMO1 (small ubiquitin-related modifier 1). The level of SUMO1 is suppressed in heart failure, and Sumo1 overexpression rescued heart failure in an animal model.\(^5\) Those experiments lead to a better understanding of SERCA2a function; however, those findings have not yet been evaluated in humans. Further elucidation of the detailed regulatory mechanism of SERCA2a would lead to novel therapeutic concepts and contribute to drug development.

Cardiomyocytes respond to external stimuli by activating signal transduction cascades by enzymatic modifications, such as phosphorylation, acetylation, ubiquitination, and glycosylation. Among them, phosphorylation has a central role in cellular signaling, and kinases can be therapeutic targets because they can be controlled by chemical compounds. MLCKs (myosin light-chain kinases) are expressed in several types of myocytes, such as smooth muscle cells, skeletal muscle cells, and cardiac myocytes, and have an important role in cardiomyocyte function, including sarcomere organization.\(^7\) SPEG (striated muscle preferentially expressed protein kinase)—a serine/threonine kinase and member of MLCKs.\(^7\) SPEG is highly expressed in the developing heart. SPEG knockout mice showed dilated cardiomyopathy–like phenotype and perinatal death.\(^7\) The JMC (junctional membrance complex) between the plasma membrane and SR is an important structure for excitation-contraction coupling in cardiomyocytes. SPEG is associated with JMC proteins, and the expression of SPEG decreases in patients with heart failure. SPEG phosphorylates the JMC protein, JPH2 (junc- tophilin-2), and is essential for JMC integrity.\(^8\) Adult-onset cardiac-specific SPEG knockout mice showed a dilated cardiomyopathy–like phenotype and died. Moreover, SPEG mutation in humans also leads to centronuclear myopathy with dilated cardiomyopathy.\(^9\) The evidence presented clearly indicates that SPEG plays a crucial role in heart homeostasis, but the precise mechanism remains elusive.

With regard to this issue, Quan et al\(^{11}\) evaluated the function of SPEG by the identification of SPEG-binding partners and focused on the physical and functional interactions between SPEG and SERCA2a. Mass spectrometry analysis revealed that SPEG is physically associated with SERCA2a. Coexpression experiments showed that SPEG augments SERCA2a function, accelerating Ca\(^{2+}\) reuptake into SR. These data suggested that SPEG would directly affect the SERCA2a function, possibly through phosphorylation. SPEG has 2 serine/threonine kinase (SK) domains in the C-terminal region. SPEG increased SERCA2a oligomerization that enhances
SPEGfl/fl/Myh6-MCM level was increased in the heart of function at around 4 weeks after tamoxifen treatment, but the SPEGfl/fl chronosomal order. They crossed the adult-onset cardiac-specific SPEG knockout mice—in weeks of age, the authors examined another mouse model—phorylation of SERCA2a Thr484 was decreased, and a dilated sosis in the heart and isolated cardiomyocytes of specific in vivo and in adult cardiomyocytes by generating cardiac-induces SERCA2a oligomerization, and enhances SERCA2a phosphotransport of Ca2+ to the SR is impaired at 4 weeks after tamoxifen treatment. Collectively, SPEG directly phosphorylates Thr484 SERCA2a, which induces SERCA2a oligomerization and SERCA2a activity enhancement. SPEG is indispensable for heart homeostasis in vivo, but SPEG is downregulated in heart failure. Moreover, SPEG may have a key role in the vicious spiral of SPEG decrement, SERCA2a functional impairment, and heart failure. Correcting impaired SPEG expression could, thus, be a therapeutic target.

Abnormality of Ca2+ homeostasis induces heart failure and fatal arrhythmia. This article showed that SPEG directly regulates the phosphorylation of Thr484 SERCA2a. Moreover, SPEG enhances the calcium reuptake activity of SERCA2a, and SPEG deficiency leads to heart failure in mice. SPEG is indispensable for heart homeostasis, but whether heart failure can be treated with SPEG gene transfer, SPEG activity augmentation, increased phosphorylation of Thr484 SERCA2a, or pThr484 SERCA2a mimic gene transfer remains unclear. Further investigation will facilitate the development of drugs to induce SPEG activity for heart failure treatment. The CUPID 2 trial failed to demonstrate the efficiency of gene therapy using adeno-associated virus/SERCA2a, but accumulating basic studies still encourage us to elucidate the undiscovered mechanisms of Ca2+ homeostasis in heart failure to perfect innovative therapies for heart failure.

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