Ca2+ Proteins in Cardiovascular Disease

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Mechanosensitive ion channels are widely expressed in the cardiovascular system. They translate mechanical forces including shear stress and stretch into biological signals. The most prominent biological signal through which the cardiovascular physiological activity is initiated or maintained are intracellular calcium ions (Ca2+). Growing evidence show that the Ca2+ entry mediated by mechanosensitive ion channels is also precisely regulated by a variety of key proteins which are distributed in the cell membrane or endoplasmic reticulum. Recent studies have revealed that mechanosensitive ion channels can even physically interact with Ca2+ regulatory proteins and these interactions have wide implications for physiology and pathophysiology.

mechanosensitive ion channels | Piezo channels | TRP channels

1. Introduction

Despite decades of efforts, cardiovascular disease is still the number one killer in the world. The latest data show that one in six elderly people dies of cardiovascular disease. In 2019, ischemic heart disease and stroke were reported to be the leading causes for disability in the age groups of 50–74 and 75 years or above [1]. As a dominant second messenger, Ca^{2+} plays an important role in the cardiovascular health and diseases. For example, dietary calcium supplement and its retention reduce cardiovascular response to sodium stress in Black people [2]. There are still some controversies over the cardiovascular effects of high Ca^{2+} intake in the diet, and whether the relationship between Ca^{2+} intake and cardiovascular disease risk is J- or U-shape [3]. Ca^{2+} is pivotal in maintaining the functions of endothelial cells, smooth muscle cells (SMCs) and cardiomyocytes. It controls the contraction or relaxation of arteries and heart and regulates blood pressure and cardiac functions [4][5][6][7].

The cell membrane controls the balance between intracellular and extracellular Ca^{2+} via various proteins, and thus maintains Ca^{2+} homeostasis. Plasma membrane Ca^{2+} ATPase (PMCA), voltage-gated calcium channel (VGCC), Na^{+}/Ca^{2+} exchanger (NCX), and Orai have been identified as the main calcium regulatory proteins on the cell membrane [8][9][10][11]. Endoplasmic reticulum (ER) as an important Ca^{2+} reservoir in the cell also contains some calcium regulatory proteins. Such proteins in the ER include stromal interaction molecules (STIM), inositol 1,4,5-trisphosphate receptor (IP3R), and sarco/endoplasmic reticulum calcium-ATPase (SERCA). All these proteins are critical in controlling cell functions, such as growth, migration, apoptosis, and metabolism [12][13][14]. As a general feedback mechanism, these key Ca^{2+} regulatory proteins are also regulated by intracellular Ca^{2+} level.

Mechanical forces are crucial for cardiovascular functions and therefore the discoveries of mechanosensitive ion channels represent a major breakthrough in understanding cardiovascular mechanobiology. In particular, the

endothelium in the cardiovascular system is subjected to regular mechanical stimulus evoked by blood flow. The discovered mechanosensitive ion channels including Piezo channels and transient receptor potential (TRP) channels can conduct the entry of cationic ions, particularly Ca^{2+}, in response to the stimulus from shear stress of blood flow [15][16]. Both Piezo and TRP channels are closely linked to the development of cardiovascular disease. In some cardiovascular diseases, such as hypertension, atherosclerosis, or aneurysmal plaques, altered mechanical stress which can directly activate mechanosensitive ion channels has been reported [17].

2. Ca^{2+} Regulatory Proteins in Cardiovascular System

2.1. NCX

NCX mainly works in the forward mode, which uses the electrochemical gradient driven by Na^+ to expel Ca^{2+} from cells in order to maintain the concentration of Ca^{2+} required for physiological activities, and the ion stoichiometric ratio is $3Na^+$:$1Ca^{2+}$ [18]. There are three types of NCX: NCX1, NCX2, and NCX3 [19]. Structural studies revealed that eukaryotic NCX protein consists of 10 transmembrane helixes. There is a large cytoplasmic regulatory loop between transmembrane helix 5 and 6. This loop includes two regulatory (Ca^{2+})-binding domains 1 and 2 [20][21], which adjust the rate of from cells to adapt to the dynamic Ca^{2+} oscillation [20][22][23]. The Ca^{2+} extrusion usually leads to smooth muscle relaxation, so that the vascular tension is reduced [24]. However, under some extreme conditions, such as high concentration of intracellular Na^+ or high positive membrane potential, NCX works in a reverse mode to evoke Ca^{2+} influx instead of the typical extrusion [22], which can then lead to contraction of SMCs and artery [25][26]. Interestingly, the forward mode of NCX can be changed to its reverse mode by the increase of cytosolic Na^+ and membrane potential [27], suggesting a feedback regulatory mechanism may exist [18].

NCX regulates many essential physiological events, such as muscle excitation-contraction or blood pressure regulation [28][29]. The deletion of NCX causes the loss of NCX function in myocardium and consequentially results in embryonic death [24]. The altered expression and regulation of NCXs could disrupt Ca^{2+} homeostasis and initiate molecular and cellular remodeling in various tissues, which is related to hypertension and heart failure. Inhibitors of NCX can improve myocardial function in patients with heart failure and bring the Ca^{2+} hyper-responsiveness back to normal in vascular SMCs from hypertensive patients [25][30]. NCX1 in smooth muscle and endothelium could play opposite roles in regulating blood pressure. Arterial blood pressure is correlated with the expression level of NCX1 in vascular SMCs [25]. The increased expression of vascular NCX1 is associated with the vasoconstriction in several animal models of salt-dependent hypertension [25]. Furthermore, reduced arterial myogenic tone and low blood pressure were observed in vascular smooth muscle NCX1 conditional knockout mice. However, for the mice with NCX1 overexpression in vascular SMCs, high blood pressure and vasoconstriction even accompanied with increased expression of transient receptor potential canonical channel (TRPC) 6 were reported [25], which suggests that NCX could control arterial constriction and regulate blood pressure by cross-talking to TRPC6 channels [25]. In the mesentery constricted by phenylephrine, antagonists of NCX reverse mode eliminate acetylcholine-evoked nitric oxide production in intact mesenteric arteries and inhibit acetylcholine- or ATP-induced increase of intracellular Ca^{2+} in cultured endothelial cells, indicating that the activation of NCX reverse mode can play an important role in mediating the acetylcholine-induced vasodilation in resistance arterial endothelial cells [31].

2.2. Orai

Orai is a highly (Ca^{2+})-selective ion channel in the plasma membrane formed by four transmembrane domains. Orai family includes Orai1, Orai2, and Orai3 [8]. The calcium release-activated calcium (CRAC) channels are composed of Orai and STIM, representing a typical voltage independent store-operated Ca^{2+} entry (SOCE) [32][33]. Store-operated Ca^{2+} channels fine tune Ca^{2+} entry in both cardiomyocytes and SMCs, and they are activated once the Ca^{2+} store in ER or sarcoplasmic reticulum (SR) is depleted or the level of cytosolic Ca^{2+} is lowered, thereby facilitating agonist-induced Ca^{2+} influx. It has also been suggested that STIM1, Orai and TRPC channels could form the molecular basis of SOCE in some types of cells and their intricate interactions control the entry of Ca^{2+} into cells to regulate numerous physiological processes [34]. Orai1 in plasma membrane and STIM1 in ER conduct Orai-STIM signaling at the membrane junction between ER and plasma membrane, and they are the bona fide molecular components of SOCE and CRAC [8][35]. Once the ER Ca^{2+} store is depleted, STIM1 protein can move to the plasma membrane and activate Orai and TRPC channels, allowing extracellular Ca^{2+} to enter the cytoplasm [8][35]. Orai2 and Orai3 channels have been discovered to be key players in regenerative Ca^{2+} oscillations induced by physiological receptor activation, while Orai1 is not necessarily involved in this process. However, the binding of Orai2 and Orai3 to Orai1 could expand the sensitivity range of receptor-activated Ca^{2+} signals [36].

Orai plays a critical role in regulating cardiovascular function in both health and disease [34][37][38]. Orai1 protein deficiency leads to heart failure in zebrafish [39]. The knockout of Orai3 in cardiomyocyte causes dilated cardiomyopathy and heart failure in mice [40]. Both Orai1 and Orai3 are the phenotype modulators of vascular SMCs. Orai1 is upregulated in SMCs during vascular injury. The downregulation of Orai1 inhibits SMC proliferation and reduces neointima formation following balloon injury of rat carotids [41]. Orai3 is also upregulated in neointimal SMCs in rat balloon injured carotid artery, and the knockdown of Orai3 inhibits neointima formation [42]. The transformation of vascular SMC phenotypes is one of the pathological characteristics in chronic hypertension, and the synergistic action between Orai and STIM mediates Ca^{2+} entry and drives the fibroproliferative gene program [43]. Orai facilitates Ca^{2+} entry and is a potential therapeutic target for the treatment of hypertension [34]. Most cardiovascular diseases are closely associated with cellular remodeling, and Ca^{2+} signaling pathways have emerged as important regulators of smooth muscle, endothelial, epithelial, platelet, and immune cell remodeling [44]. Ca^{2+}-permeable Orai channel is also important for endothelial cell proliferation and angiogenesis [45][46]. In terms of vascular physiology and functional regulation, Orai1 appears to trigger the increase of vascular permeability, which is an early marker of atherogenesis. Knockdown of Orai1 reduces the histone 1-induced hyperpermeability in endothelial cells [47]. ApoE knockout mice are a common model for atherosclerosis. A high fat diet can upregulate the expression of Orai1 mRNA and protein in aortic tissue. SiRNA knockdown of Orai1 can reduce the size of atherosclerotic plaque [48]. The migration of neutrophil is another hallmark in atherosclerosis, and during this process Orai1 is required for neutrophil migration to the inflammatory endothelium [44]. All these experimental evidence show that Orai1 expression is associated with development of atherogenesis. Moreover, Orai often acts in conjunction with STIM to form CRAC, which can be responsible for many physiological functions or the development of various cardiovascular diseases [34][38][49].

2.3. STIM

STIM is a single pass transmembrane protein residing in the ER. It contains two homologous proteins, STIM1 and STIM2 [50][51]. The function of STIM is to sense the concentration of Ca^{2+} in ER and makes appropriate response through conformational change to regulate Ca^{2+} homeostasis [38][52]. STIM1 stays at a closed state when ER lumen is filled with Ca^{2+} and transitions to an open state when Ca^{2+} in the ER lumen is decreased [53]. The Ca^{2+}-sensitive domain in the STIM N-terminal senses Ca^{2+} level of ER ranging from 100 to 400 μM [51][54], and the C-terminal of STIM interacts with Orai to form CRAC channel to induce Ca^{2+} influx [51][55]. As mentioned above, the interactions between STIM1 and Orai1 regulate physiological and pathological functions [34][38][49].

STIM is involved in both the cardiac physiological functions and the cardiac disease development. STIM is essential for the maintenance of myocardial contractility, and its knockout leads to a reduction in left ventricular contractility. STIM1 is expressed more abundantly in early cardiomyocytes than in somatic cells. Cardiomyocyte-STIM1-specific knockout mice exhibit dilated cardiomyopathy and cardiac fibrosis with increased stress biomarkers and altered organelle morphology in the heart, suggesting that STIM1 can regulate myocardial development and heart function [38]. However, the spatially differential distribution of STIM1-triggered Ca^{2+} signaling generates the Ca^{2+} microdomain that regulates myofilament remodeling and activates pro-hypertrophic factors locally, and as a consequence, pathological cardiac hypertrophy is induced [56]. The STIM1-guided Ca^{2+} signaling is also involved in thrombosis. The aggregation of platelets at the site of thrombosis requires the increase of intracellular Ca^{2+} concentration. STIM1 is involved in this process through maintaining a high Ca^{2+} concentration. In addition, STIM1 stabilizes the thrombus by promoting the expression of phosphatidylserine in plasma membrane [38][57]. The upregulation of STIM induces fibroproliferative gene expression and vascular SMC remodeling, which eventually leads to chronic hypertension [43]. The cell proliferation and migration promoted by STIM1 are also involved in atherosclerosis [58][59]. Oxidized low-density lipoprotein (ox-LDL) can increase the expression of STIM1, and then promote cell proliferation and migration in mouse aortic SMCs. Silencing STIM1 inhibits ox-LDL-induced cell proliferation and migration and hence suppresses atherosclerosis [58][59]. The role of STIM1 in the pathogenesis of these diseases suggests that specific inhibition of STIM1 may contribute to the treatment of these diseases. STIM2 is another important protein for health. Studies on STIM2 deficient mice show that they gradually die from 4 to 8 weeks [60].STIM2 has similar functional effects to STIM1 in some aspects. Both proteins can promote vascular remodeling by inducing the transformation of phenotypes in pulmonary artery SMCs [61].

2.4. IP3R

IP3R is a tetrameric channel consisting of four glycoproteins in the ER or SR. So far, three types of IP3R channels have been identified: IP3R1, IP3R2, and IP3R3 [62]. IP3R has four structural regions: IP3 binding region, central regulatory region, transmembrane domain, and C-terminal region. It can be activated by the selective ligand inositol 1,4,5-triphosphate (IP3) and is permeable to Ca^{2+} [63]. All these isoforms of IP3R can be expressed in vascular SMCs. They are important for the physiological functioning of the cardiovascular system [64]. IP3R is one of the major sources for intracellular Ca^{2+} release. The overexpression of IP3R enhances ER Ca^{2+} depletion, which reduces ER intraluminal Ca^{2+} concentration in the vicinity of STIM1 and then activates Orai ion channels [65]. In response to increased IP3 or decreased Ca^{2+} in ER, IP3Rs empty Ca^{2+} stored in the ER and activate Ca^{2+} inward flow [66]. IP3R also functions on the membrane contact sites between ER and mitochondria to transport

Ca^{2+} from ER to mitochondria. Each isoform of IP3R can mediate this contact and Ca^{2+} transport, but IP3R2 is the most efficient one in delivering Ca^{2+} to mitochondria from ER [67][68]. The voltage-dependent anion channel on the outer mitochondrial membrane can also enhance Ca^{2+} accumulation through physical interaction with IP3R [69], which is vital for the maintenance of mitochondrial function.

Under physiological conditions, IP3R signal controls the contraction, migration, and proliferation of vascular SMCs. However, under the pathological conditions, IP3R is involved in the development of atherosclerosis and hypertension [70]. IP3R is activated following the stimulation of G-protein coupled receptors and binds to STIM1 upon Ca^{2+} depletion in ER. The association of IP3R-STIM1 increases Ca^{2+} inward flow [65]. When IP3 binds with IP3Rs, vasoconstriction and hypertension can be induced as a consequence to the increased concentration of cytoplasmic Ca^{2+} released from the ER. The deletion of IP3Rs reduces the contractile response to vasoconstrictors and even reverses the pathological states [64]. In the heart, IP3R-mediated Ca^{2+} release ensures the integrity of cardiac excitation-contraction coupling, which forms the basis of the heartbeat [71]. The dysfunction of IP3R in cardiomyocytes leads to the disturbance of local Ca^{2+} homeostasis, which is closely related to congenital diseases, increased risk of arrhythmia, decreased contractility, or heart failure related arrhythmias [72]. The expression of IP3R is upregulated in atrial fibrillation, and inhibition of IP3R can significantly reduce the occurrence and duration of atrial fibrillation. Therefore, IP3R may emerge as a new target for the treatment of atrial fibrillation [73].

2.5. SERCA

SERCA is a Ca^{2+} transporter located on SR/ER and is mainly responsible for the transport of cytoplasmic Ca^{2+} back to SR/ER. SERCA isoform is encoded by SERCA1, SERCA2, or SERCA3 genes. Each isoform may have differential roles in different tissues or cells [74]. There are four functional domains (M, N, P, and A) and a polypeptide chain in SERCA protein. The M domain contains transmembrane components and Ca^{2+} binding sites, while N, P and A located in the sarcoplasm are responsible for ATP hydrolysis [75]. Each ATP hydrolysis can transport 2 Ca^{2+} to the ER lumen in exchange for 1 H^{+} [76]. SERCA2a is the major isoform of cardiac SERCA, while SERCA2b is the major one of vascular SERCA.

The influx of Ca^{2+} into SR/ER mediated by SERCA2 is necessary for the relaxation of cardiomyocytes and blood vessels. The disruption of SERCA2 activity leads to ER stress and cardiovascular disease [75]. Hormones, phospholamban and sarcolipin are the common regulators of SERCA. Especially, phospholamban plays a major role in regulating SERCA, and its interaction with SERCA2a reduces the binding affinity of SERCA2a to Ca^{2+} at low cytoplasmic Ca^{2+} concentration [77]. The downregulation of SERCA2a is found in failing heart and atherosclerotic vessels [78]. The decreased protein level of SERCA2a and p16-phospholamban leads to left ventricular diastolic dysfunction and elevated arterial blood pressure [79]. Activation of SERCA can accelerate the reuptake of Ca^{2+} by SR, which would improve the diastolic dysfunction of myocardium, and result in strong antiarrhythmic effect [80][81]. Our groups found that the *S*-glutathiolation of the amino acid residue Cys674 (C674) is key to the increase of the activity in SERCA2 under physiological conditions [82][83], but this post-translational protein modification is prevented by the irreversible oxidation of C674 thiol in pathology hallmarked by high level of ROS, including atherosclerosis, aortic aneurysms, aging and hypertension [82][84][85][86]. The substitution of the SERCA2 C674 by

serine causes impaired angiogenesis following hindlimb ischemia by interrupting the physiological functions of endothelial cells and macrophage [87][88], increases blood pressure by inducing sodium retention and ER stress in the kidney [86], exacerbates angiotensin II-induced aortic aneurysm by switching the phenotypes in aortic SMCs [89], aggravates high fat diet-induced aortic atherosclerosis by evoking inflammatory response in endothelial cells and macrophage (our unpublished data), promotes pulmonary vascular remodeling, and protects against left ventricular dilation caused by chronic ascending aortic constriction [90]. All these data imply that the redox state of C674 and the function of SERCA2 are critical to the maintenance of cardiovascular homeostasis.

Currently, there are some ongoing clinical trials for the drugs specifically targeting these Ca^{2+} regulatory proteins in the cardiovascular system, as shown in **Table 1**. These trials provide promising opportunities for the treatment of cardiovascular diseases.

Table 1. Current clinical trials for drugs targeting these Ca^{2+} regulatory proteins in the cardiovascular system according to the ClinicalTrials.gov website (available online and accessed on 12 August 2021).

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Ca^{2+} Regulatory Proteins	Treatment	Cardiovascular Disease	Phase
SERCA	AAV1/SERCA2a (MYDICAR) [91]	Ischemic cardiomyopathy; non-ischemic cardiomyopathy; heart failure; cardiomyopathies	Phase 2
	MYDICAR-single intracoronary infusion [92]	Heart failure, congestive; ischemic cardiomyopathy; non-ischemic cardiomyopathy	Phase 2
	MYDICAR [93]	Chronic heart failure	Phase 2
	SRD-001 [94]	Congestive and systolic heart failure	Phase 1/Phase 2
	Istaroxime [95]	Heart failure [96]	Early phase 1
NCX	MYDICAR [97]	Chronic heart failure	Phase 2
Orai	No resource	No resource	No resource
STIM	No resource	No resource	No resource
IP3R	No resource	No resource	No resource

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