CaMKII: The molecular villain that aggravates cardiovascular disease (Review)

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Abstract. Pathological remodeling of the myocardium is an integral part of the events that lead to heart failure (HF), which involves altered gene expression, disturbed signaling pathways and altered Ca^{2+} homeostasis and the players involved in this process. Of particular interest is the chronic activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) isoforms in heart, which further aggravate the injury to myocardium. Expression and activity of CaMKII have been found to be elevated in various conditions of stressed myocardium and in different heart diseases in both animal models as well as heart patients. CaMKII is a signaling molecule that regulates many cellular pathways by phosphorylating several proteins involved in excitation-contraction coupling and relaxation events in heart, cardiomyocyte apoptosis, transcriptional activation of genes related to cardiac hypertrophy, inflammation, and arrhythmias. CaMKII is activated by reactive oxygen species (ROS), which are elevated under conditions of ischemia-reperfusion injury and in a cyclical manner, CaMKII in turn elevates ROS production. Both ROS and activated CaMKII increase Ca-induced Ca release from sarcoplasmic reticulum, which leads to cardiomyocyte membrane depolarization and arrhythmias. These CaMKII-mediated changes in heart ultimately culminate in dysfunctional myocardium and HF. Genetic studies in animal models clearly demonstrated that inactivation of CaMKII is protective against a variety of stress induced cardiac dysfunctions. Despite significant leaps in understanding the structural details of CaMKII, which is a very complicated and multimeric modular protein, currently there is no specific and potent inhibitor of this enzyme, that can be developed for therapeutic purposes.

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Contents

1. Introduction

Advances in medicine over the past few decades significantly lowered cardiovascular disease-linked mortality by up to 75% and increased the survival rate of patients with cardiac disease, but at the same time this has led to a great increase in the number of people surviving with injured heart (1). However, the increasing incidence of obesity, and associated hypertension and diabetes coupled with unhealthy lifestyles is causing a significant increase in the number of surviving individuals with heart disease, adding burden on the society in terms of health, economy and productivity (2,3). Several diseases and metabolic disturbances can be contributed to heart failure (HF) and these include myocardial infarction (MI), hypertension, valvular disease, genetic disorders, diabetes and obesity. HF occurs because of the compromised ability of myocardium to exert systolic contraction with enough force to pump blood; with characteristic reduced ejection fraction or it can be due to lowered diastolic filling, but with the preservation of ejection fraction. While acute HF is the sudden appearance of HF symptoms such as congestion and difficulty to breath (4), chronic HF is marked by the inability of heart to function optimally over an extended period of time (4,5).

Pathological remodeling of the myocardium is an integral part of the HF syndromes (6), which involves altered gene expression and disturbed signaling pathways and altered contractile response of myocardium. Of particular significance are the changes in the functionality of proteins that play a central part in intracellular Ca^{2+} handling as well as ion channels involved in Ca^{2+} transport. Cardiac muscle contraction is dependent on the maintenance of Ca^{2+} homeostasis, which is essential for excitation-contraction (E-C) coupling of cardiomyocyte. Thus, electrical depolarization of the cardiomyocyte membrane swiftly moves to the center of

the cell via the network of transverse tubules (t-tubules), which terminate close to sarcoplasmic reticulum (SR), with a 12 nm gap. Membrane depolarization triggers the rapid diffusion of extracellular Ca^{2+} to the SR, through these gaps, facilitated by the L-type Ca^{2+} channels (LTCC). This influx of Ca^{2+} leads to Ca^{2+}-induced calcium release (CICR) from the SR through the type 2-ryanodine receptor (RyR2). This elevated calcium promotes cross-bridge cycling by relieving actin from troponin C-dependent inhibition, thereby causing cardiomyocyte contraction. Then, Ca^{2+} is taken back into the SR lumen by the SR Ca^{2+} ATPase 2a (7). Considering the significance of Ca^{2+} in heart muscle contraction, it is appreciated that disturbances in the Ca^{2+} handling machinery in cardiomyocyte can potentially lead to HF. Thus HF is characterized by disturbed Ca^{2+} leak from SR, mediated by RyR2, even though the precise mechanisms are not clear (8). Other players such as SERCA are also deranged in HF. Besides membrane depolarization, intracellular Ca^{2+} is important in many other cell processes including oxidative stress, mitochondrial function, apoptosis and autophagy.

2. Ca^{2+}/calmodulin-dependent protein kinase II

Several studies have indicated that a Ca^{2+}-regulated protein kinase, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) plays a critical role in E-C coupling, contractility of cardiomyocyte (9,10), mitochondrial function and cardiomyocyte survival (11,12). Expression and activity of CaMKII have been found to be elevated in various conditions of stressed myocardium and in different heart diseases in both animal models as well as heart patients (9-14). The activation of CaMKII can be either at the level of this enzyme protein itself or at an upstream signaling event involving catecholamines (15) or renin-angiotensin-aldosterone systems (16). Abnormally elevated CaMKII activity can cause dysfunction of several downstream events whose components are regulated by CaMKII, such as E-C coupling, structural remodeling, and transcriptional activation of certain inflammatory proteins and apoptosis (17).

Structure/function features of CaMKII. CaMKII is a serine/threonine kinase with a broad range of protein substrates and wide tissue distribution. There are 4 isoforms of CaMKII (α, β, δ and γ), coded for by 4 separate genes, with heart expressing predominantly the δ-isoform, with some γ-isoform as well. Alternate splicing of mRNA adds further complexity to the CaMKII isoforms and their function and regulation (18). There are three main domains in the CaMKII monomer-N-terminal catalytic domain, regulatory domain and the C-terminal association domain (Fig. 1) (19). The regulatory domain, which interacts with the catalytic site, maintains the catalytic activity low under unstimulated basal conditions, and contains binding sites for Ca^{2+} and calmodulin. The C-terminal association domain participates in the multimerization process, thus forming the mature dodecameric holoenzyme, with two hexameric stacked rings (20). Complex of calmodulin and Ca^{2+} binds with the regulatory domain and this displaces this domain from the catalytic domain thereby restoring the activity of the enzyme (activation) and also exposes certain other regulatory binding sites, which can influence the CaMKII activity. CaMKII can phosphorylate several proteins involved in Ca^{2+} homeostasis, the well-studied protein targets being LTCC, RyR2, voltage-gated Na^+ channel and K^+ channels (21,22) and also ATP-sensitive K^+ channels (23) and chloride channels (24), which have been shown to be important for cardiac arrhythmias. Regulation and activity level of CaMKII depends upon its holoenzymic state and post-translational modifications including phosphorylation, glycosylation and oxidation. CaMKII is known to autophosphorylate itself at Thr286/287 residue of the calmodulin-Ca^{2+} bound catalytic domain, mediated by another adjacent catalytic domain. This autophosphorylation renders the catalytic domain to maintain its activity even in the absence of calmodulin and Ca^{2+} (25,26).

Organization of CaMKII in cardiomyocytes. Subcellular localization is critical for the maintenance of membrane excitability and CaMKII is found to be distributed in high density near the t-tubules of cardiomyocyte, close to LTCC (Cav1.2) and to RyR2 channels of SR, which regulate the Ca-induced Ca release intracellularly (Fig. 2). Thus, phosphorylation of S2814 of RyR2 by CaMKII leads to dysregulated intracellular Ca^{2+} homeostasis, which in turn cause perturbation of maladaptive stress response and proarrhythmic events, thus further aggravating the HF (Figs. 1 and 2). Thus, mouse models which express RyR2 with S2814A mutation and thus are not phosphorylated by CaMKII, are protected from pressure overload *in vivo* (27). CaMKII is also found in mitochondria, nucleus and near the intercalated disc (17). CaMKII subcellular localization appears to be dependent on the nature of the target and its location and the presence of interacting domains on the target. Thus α- and β-subunits of LTCC, which are phosphorylated by CaMKII, bind with CaMKII, because of the homology between the phosphorylation sites and the auto-inhibitory region of the CaMKII (21,28). A similar homology domain, as seen in the LTCC β-subunit, is also found in the actin-associated protein, βIV-spectrin, to which CaMKII is known to bind. This interaction is a prerequisite for the CaMKII-mediated phosphorylation of the voltage-gated Na^+ channels at the intercalated disc in cardiomyocytes (29).

3. Evidence for CaMKII as a therapeutic target in heart disease

CaMKII acts as a molecular nexus that connects neurohumoral stimulation to HF and cardiac remodeling (20). There has been a significant development in our understanding of the role of CaMKII in cardiovascular diseases and several reports over the past two decades have suggested such roles, making CaMKII a potential therapeutic target. Thus, it has been noted that cytosolic CaMKIIδC isoform as well as the nuclear CaMKIIδB isoform were found to be elevated in the two ventricles of patients with ischemic cardiomyopathy (30). There is also a significant elevation of autonomous activity of CaMKII and its expression, in patients with advanced and end stage HF (31). As the upregulation of CaMKII is associated with heart disease and failure by promoting apoptosis, inflammation that leads to cardiac dysfunction (32,33), the possibility that inhibition of this enzyme activity can have therapeutic effects has been considered. Experimental transgenic animal models, overexpressing CaMKII have been

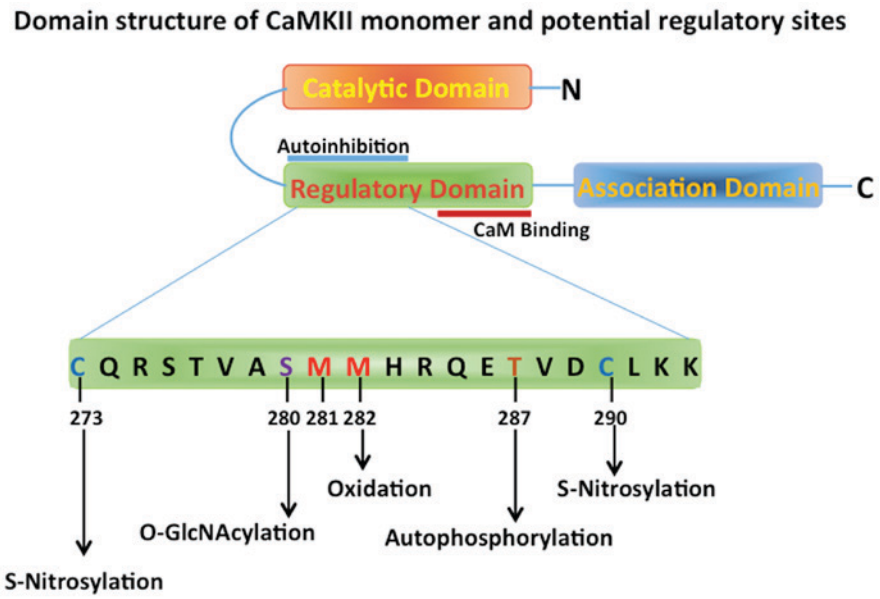

Figure 1. Domain structure of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) monomer and potential regulatory sites. A schematic showing the CaMKII monomer with catalytic, regulatory and association domains. Catalytic domain function is normally obscured by the regulatory domain. Association domain is instrumental in forming the holoenzyme that consists of 12 monomers. Post-translational modifications by different stress stimuli and neurohormonal signaling at the indicated sites in the regulatory domain lead to sustained activation of CaMKII by relieving the catalytic domain.

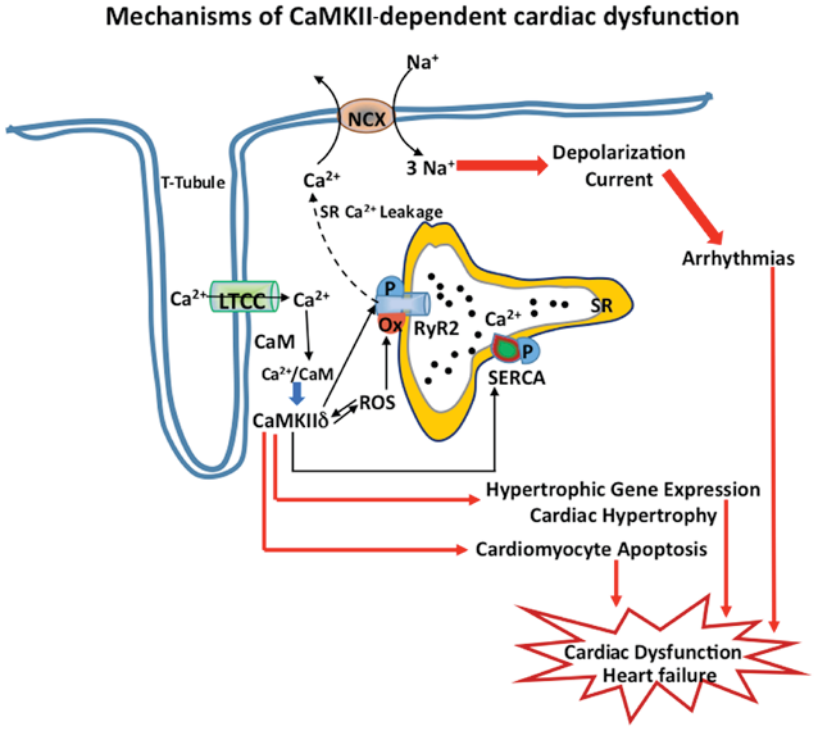

Figure 2. Mechanisms of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) dependent cardiac dysfunction. Increase in intracellular Ca^{2+} through L-type Ca^{2+} channels, leads to CaMKIIδ activation via Ca^{2+}/Calmodulin (CaM) in cardiomyocyte. Activated CaMKIIδ contributes to elevated reactive oxygen species (ROS), which can also arise from other stress stimuli, themselves can activate CaMKIIδ, resulting in a vicious cycle of CaMKIIδ activation. Both the phosphorylation mediated by CaMKIIδ and ROS-mediated oxidation of the type 2-ryanodine receptor (RyR2) in sarcoplasmic reticulum (SR) lead to enhanced SR Ca^{2+} load and in turn, cause SR Ca^{2+} leak followed by the re-uptake of Ca^{2+} by SERCA into SR. This triggers sodium/calcium exchanger (NCX)-dependent depolarizing current (transient inward current), which contributes to arrhythmia. Activated CaMKIIδ also elevates transcription of cardiac hypertrophy genes, which culminates in cardiac hypertrophy and thus dysfunction of heart. Activated CaMKII is known to trigger cardiomyocyte apoptosis program, leading to loss of cardiomyocytes and thus damaged myocardium. All these events resulting from CaMKII activation, contribute to failure of the heart.

found to suffer from HF (34), whereas CaMKII knockout mice were protected from HF induced by transaortic constriction (35). Additionally, mice expressing a mutant CaMKII (S2814D), which is constitutively active, suffered exacerbated mortality (36). CaMKIIδγ knockout mice with total deletion of heart specific isoforms CaMKII, are protected

from pressure overload and β-adrenergic stimulation-induced cardiac dysfunction and interstitial fibrosis (32,37). Similarly, the elevated activity of CaMKII is also associated with atrial fibrillation and sinus node disease (38) and several other HF contributory diseases such as inherited arrhythmias (39,40).

Oxidation of 281/282 methionine residue in CaMKII is susceptible to oxidative stress and this oxidation leads to the activation of CaMKII and it has been shown that this oxidation is particularly important in cardiomyocytes as it may relate to conditions of ischemia/reperfusion injury (41). Met281/282 oxidation prevents the re-association of the inhibitory regulatory domain with the catalytic domain of CaMKII (Fig. 1) (42). Angiotensin II and aldosterone are shown to mediate their activation effects on CaMKII via oxidation (43), as cardiomyocytes expressing oxidation-resistant mutant CaMKII were protected from angiotensin II-induced apoptosis (41). Also, diabetic mice expressing an oxidation-resistant CaMKII mutant (MM281/282VV) were found to be protected from MI (44). In fact, it has been noted that increased oxidation status of CaMKII seen after MI in diabetic patients appears to be associated with higher mortality, than in non-diabetic individuals, which again emphasizes the detrimental effects of CaMKII activation, particularly when the heart is stressed.

Of note, CaMKII oxidation and activity is found to be much less following MI in mice with deletion of the *MyD88* gene, an important mediator of inflammatory signaling. These MyD88-knockout mice also show lower post MI inflammatory cell infiltration, cardiomyocyte death and fibrosis. Oxidized CaMKII can in turn enhance the transcription of proinflammatory genes by enhancing NF-κB activity (45). Other post-translational modifications of CaMKII that cause its activation and are involved in the pathology of HF include nitrosylation and O-GlcNAcylation, which are important under hyperglycemic conditions seen in diabetes (46).

4. Therapeutic measures against CaMKII

Inasmuch as the activation of CaMKII is involved with heart disease, several studies have focused on developing CaMKII inhibitors that have the potential to have therapeutic effects in HF and heart diseases. Most of the currently available inhibitors are for research purposes and lack specificity and/or potency. For example, KN-93, which is a commonly used CaMKII inhibitor, also directly affects many other ion channel including LTCC (47). Administration of KN-93 to mice with structural heart disease, for 3 weeks led to chronic inhibition of CaMKIIδ and resulted in a dose-dependent improvement in left ventricular function (48). Similarly, peptide molecules (AIP and AC3-I) that mimic the autoinhibitory-regulating domain of CaMKII, also have several limitations regarding their specificity and delivery. Among the several inhibitors tested, the most promising is the endogenous inhibitor, known as CaMKIIN and its derivatives, which bind to the active kinase at the B/C sites, which also prevent protein-protein interactions of CaMKII with other targeting proteins (47). It has been recently shown that targeting CaMKII/ ERK interaction in heart muscle using selective CaMKII peptide inhibitor AntCaNtide was able to prevent hypertrophy in spontaneously hypertensive rats (49). The first generation CaMKII inhibitors based on targeting the ATP binding to catalytic site and the recent availability of crystal structures of CaMKII holoenzyme both in its autoinhibited as well as active states may be useful in the development of more specific and potent inhibitors for this enzyme. Furthermore, blockade of activating pathways such as O-GlcNAc modification were also found to be effective in preventing arrhythmogenesis in diabetic animals by inhibiting the hexosamine biosynthetic pathway using the inhibitor DON (50). Thus, there are formidable difficulties in achieving the required specificity for developing CaMKII inhibitors that can be developed for therapeutic applications (47).

In addition to pharmacological inhibitors, exercise, which has proven beneficial cardiovascular effects, seems to have the ability to antagonize the negative effects of CaMKIIδ in failing heart. Thus, aerobic training caused a reduction in CaMKIIδ activity and improved heart function in diabetic mice compared to non-exercising diabetic mice (51). Notably, it has been demonstrated that swimming exercise may obliterate the O-GlcNAcylation-mediated activation of CaMKII in type I diabetic mice with the resultant improvement in heart condition (52). Thus, of note is along with pharmacological approaches, lifestyle changes can be beneficial in protecting from the CaMKII-mediated aggravation of injured or stressed heart. As such, the development of specific drugs that target heart isoforms of CaMKII seems a far-reaching goal and further work is needed in understanding structure-activity relationships of these isoenzymes to accomplish this task.

5. Conclusions

HF involves altered gene expression, disturbed signaling pathways and altered Ca^{2+} homeostasis. Chronic activation of CaMKII isoforms in heart further aggravates the injury to myocardium and the expression and activity of CaMKII is elevated in myocardium in different heart diseases and stress conditions. CaMKII regulates many cellular pathways such as E-C coupling and relaxation events in heart, cardiomyocyte apoptosis, transcriptional activation of genes related to cardiac hypertrophy, inflammation, and arrhythmias. CaMKII and reactive oxygen species, which mutually activate each other, increase CICR from SR, which leads to cardiomyocyte membrane depolarization and arrhythmias. All these CaMKII-mediated changes in heart ultimately culminate in dysfunctional myocardium and HF. Despite significant leaps in understanding the structural details of CaMKII, which is a very complicated and multimeric modular protein, and genetic studies implicating CaMKII in the pathogenesis of HF, currently there is no specific and potent inhibitor of this enzyme, that can be developed for therapeutic purposes and further study is needed in this direction.

References

1. Nabel EG and Braunwald E: A tale of coronary artery disease and myocardial infarction. N Engl J Med 366: 54-63, 2012.
2. Daniels L, Bell JR, Delbridge LM, McDonald FJ, Lamberts RR and Erickson JR: The role of CaMKII in diabetic heart dysfunction. Heart Fail Rev 20: 589-600, 2015.
3. Mathers CD and Loncar D: Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 3: e442, 2006.
4. Joseph SM, Cedars AM, Ewald GA, Geltman EM and Mann DL: Acute decompensated heart failure: Contemporary medical management. Tex Heart Inst J 36: 510-520, 2009.

5. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, *et al*; ESC Committee for Practice Guidelines: Esc guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The task force for the diagnosis and treatment of acute and chronic heart failure 2012 of the European Society of Cardiology. Developed in collaboration with the heart failure association (hfa) of the esc. Eur Heart J 33: 1787-1847, 2012.
6. Hill JA and Olson EN: Cardiac plasticity. N Engl J Med 358: 1370-1380, 2008.
7. Bers DM: Cardiac sarcoplasmic reticulum calcium leak: Basis and roles in cardiac dysfunction. Annu Rev Physiol 76: 107-127, 2014.
8. Cho GW, Altamirano F and Hill JA: Chronic heart failure: Ca(2+), catabolism, and catastrophic cell death. Biochim Biophys Acta 1862: 763-777, 2016.
9. van Oort RJ, Brown JH and Westenbrink BD: CaMKII confirms its promise in ischaemic heart disease. Eur J Heart Fail 16: 1268-1269, 2014.
10. Grandi E, Edwards AG, Herren AW and Bers DM: CaMKII comes of age in cardiac health and disease. Front Pharmacol 5: 154, 2014.
11. Bers DM: Ca^{2+}-calmodulin-dependent protein kinase II regulation of cardiac excitation-transcription coupling. Heart Rhythm 8: 1101-1104, 2011.
12. Couchonnal LF and Anderson ME: The role of calmodulin kinase II in myocardial physiology and disease. Physiology (Bethesda) 23: 151-159, 2008.
13. Luczak ED and Anderson ME: CaMKII oxidative activation and the pathogenesis of cardiac disease. J Mol Cell Cardiol 73: 112-116, 2014.
14. Bell JR, Vila-Petroff M and Delbridge LM: CaMKII-dependent responses to ischemia and reperfusion challenges in the heart. Front Pharmacol 5: 96, 2014.
15. Grimm M, Ling H and Brown JH: Crossing signals: Relationships between β-adrenergic stimulation and CaMKII activation. Heart Rhythm 8: 1296-1298, 2011.
16. Zhao Z, Fefelova N, Shanmugam M, Bishara P, Babu GJ and Xie LH: Angiotensin II induces afterdepolarizations via reactive oxygen species and calmodulin kinase II signaling. J Mol Cell Cardiol 50: 128-136, 2011.
17. Swaminathan PD, Purohit A, Hund TJ and Anderson ME: Calmodulin-dependent protein kinase II: Linking heart failure and arrhythmias. Circ Res 110: 1661-1677, 2012.
18. Mishra S, Gray CB, Miyamoto S, Bers DM and Brown JH: Location matters: Clarifying the concept of nuclear and cytosolic CaMKII subtypes. Circ Res 109: 1354-1362, 2011.
19. Hoelz A, Nairn AC and Kuriyan J: Crystal structure of a tetradecameric assembly of the association domain of Ca^{2+}/calmodulin-dependent kinase II. Mol Cell 11: 1241-1251, 2003.
20. Anderson ME, Brown JH and Bers DM: CaMKII in myocardial hypertrophy and heart failure. J Mol Cell Cardiol 51: 468-473, 2011.
21. Bers DM and Morotti S: Ca(2+) current facilitation is CaMKII-dependent and has arrhythmogenic consequences. Front Pharmacol 5: 144, 2014.
22. Mustroph J, Maier LS and Wagner S: CaMKII regulation of cardiac K channels. Front Pharmacol 5: 20, 2014.
23. Sierra A, Zhu Z, Sapay N, Sharotri V, Kline CF, Luczak ED, Subbotina E, Sivaprasadarao A, Snyder PM, Mohler PJ, *et al*: Regulation of cardiac ATP-sensitive potassium channel surface expression by calcium/calmodulin-dependent protein kinase II. J Biol Chem 288: 1568-1581, 2013.
24. Sellers ZM, De Arcangelis V, Xiang Y and Best PM: Cardiomyocytes with disrupted CFTR function require CaMKII and Ca(2+)-activated Cl(-) channel activity to maintain contraction rate. J Physiol 588: 2417-2429, 2010.
25. Braun AP and Schulman H: The multifunctional calcium/calmodulin-dependent protein kinase: From form to function. Annu Rev Physiol 57: 417-445, 1995.
26. Dupont G, Houart G and De Koninck P: Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations: A simple model. Cell Calcium 34: 485-497, 2003.
27. Respress JL, van Oort RJ, Li N, Rolim N, Dixit SS, deAlmeida A, Voigt N, Lawrence WS, Skapura DG, Skårdal K, *et al*: Role of RyR2 phosphorylation at S2814 during heart failure progression. Circ Res 110: 1474-1483, 2012.
28. Anderson ME: Sticky fingers: CaMKII finds a home on another ion channel. Circ Res 104: 712-714, 2009.
29. Makara MA, Curran J, Little SC, Musa H, Polina I, Smith SA, Wright PJ, Unudurthi SD, Snyder J, Bennett V, *et al*: Ankyrin-G coordinates intercalated disc signaling platform to regulate cardiac excitability in vivo. Circ Res 115: 929-938, 2014.
30. Sossalla S, Fluschnik N, Schotola H, Ort KR, Neef S, Schulte T, Wittköpper K, Renner A, Schmitto JD, Gummert J, *et al*: Inhibition of elevated Ca^{2+}/calmodulin-dependent protein kinase II improves contractility in human failing myocardium. Circ Res 107: 1150-1161, 2010.
31. Maier LS: CaMKIIdelta overexpression in hypertrophy and heart failure: Cellular consequences for excitation-contraction coupling. Braz J Med Biol Res 38: 1293-1302, 2005.
32. Weinreuter M, Kreusser MM, Beckendorf J, Schreiter FC, Leuschner F, Lehmann LH, Hofmann KP, Rostosky JS, Diemert N, Xu C, *et al*: CaM kinase II mediates maladaptive post-infarct remodeling and pro-inflammatory chemoattractant signaling but not acute myocardial ischemia/reperfusion injury. EMBO Mol Med 6: 1231-1245, 2014.
33. Mattiazzi A, Bassani RA, Escobar AL, Palomeque J, Valverde CA, Vila Petroff M and Bers DM: Chasing cardiac physiology and pathology down the CaMKII cascade. Am J Physiol Heart Circ Physiol 308: H1177-H1191, 2015.
34. Zhang T, Maier LS, Dalton ND, Miyamoto S, Ross J Jr, Bers DM and Brown JH: The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. Circ Res 92: 912-919, 2003.
35. Backs J, Backs T, Neef S, Kreusser MM, Lehmann LH, Patrick DM, Grueter CE, Qi X, Richardson JA, Hill JA, *et al*: The delta isoform of CaM kinase II is required for pathological cardiac hypertrophy and remodeling after pressure overload. Proc Natl Acad Sci USA 106: 2342-2347, 2009.
36. van Oort RJ, McCauley MD, Dixit SS, Pereira L, Yang Y, Respress JL, Wang Q, De Almeida AC, Skapura DG, Anderson ME, *et al*: Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. Circulation 122: 2669-2679, 2010.
37. Kreusser MM, Lehmann LH, Keranov S, Hoting MO, Oehl U, Kohlhaas M, Reil JC, Neumann K, Schneider MD, Hill JA, *et al*: Cardiac CaM kinase II genes δ and γ contribute to adverse remodeling but redundantly inhibit calcineurin-induced myocardial hypertrophy. Circulation 130: 1262-1273, 2014.
38. Wu Y and Anderson ME: CaMKII in sinoatrial node physiology and dysfunction. Front Pharmacol 5: 48, 2014.
39. DeGrande S, Nixon D, Koval O, Curran JW, Wright P, Wang Q, Kashef F, Chiang D, Li N, Wehrens XH, *et al*: CaMKII inhibition rescues proarrhythmic phenotypes in the model of human ankyrin-B syndrome. Heart Rhythm 9: 2034-2041, 2012.
40. Liu N, Ruan Y, Denegri M, Bachetti T, Li Y, Colombi B, Napolitano C, Coetzee WA and Priori SG: Calmodulin kinase II inhibition prevents arrhythmias in RyR2(R4496C+/-) mice with catecholaminergic polymorphic ventricular tachycardia. J Mol Cell Cardiol 50: 214-222, 2011.
41. Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, *et al*: A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell 133: 462-474, 2008.
42. Chao LH, Pellicena P, Deindl S, Barclay LA, Schulman H and Kuriyan J: Intersubunit capture of regulatory segments is a component of cooperative CaMKII activation. Nat Struct Mol Biol 17: 264-272, 2010.
43. Velez Rueda JO, Palomeque J and Mattiazzi A: Early apoptosis in different models of cardiac hypertrophy induced by high renin-angiotensin system activity involves CaMKII. J Appl Physiol 1985 112: 2110-2120, 2012.
44. Luo M, Guan X, Luczak ED, Lang D, Kutschke W, Gao Z, Yang J, Glynn P, Sossalla S, Swaminathan PD, *et al*: Diabetes increases mortality after myocardial infarction by oxidizing CaMKII. J Clin Invest 123: 1262-1274, 2013.
45. Singh MV, Swaminathan PD, Luczak ED, Kutschke W, Weiss RM and Anderson ME: MyD88 mediated inflammatory signaling leads to CaMKII oxidation, cardiac hypertrophy and death after myocardial infarction. J Mol Cell Cardiol 52: 1135-1144, 2012.
46. Mollova MY, Katus HA and Backs J: Regulation of CaMKII signaling in cardiovascular disease. Front Pharmacol 6: 178, 2015.
47. Pellicena P and Schulman H: CaMKII inhibitors: From research tools to therapeutic agents. Front Pharmacol 5: 21, 2014.

48. Zhang R, Khoo MS, Wu Y, Yang Y, Grueter CE, Ni G, Price EE Jr, Thiel W, Guatimosim S, Song LS, *et al*: Calmodulin kinase II inhibition protects against structural heart disease. Nat Med 11: 409-417, 2005.
49. Cipolletta E, Rusciano MR, Maione AS, Santulli G, Sorriento D, Del Giudice C, Ciccarelli M, Franco A, Crola C, Campiglia P, *et al*: Targeting the CaMKII/ERK interaction in the heart prevents cardiac hypertrophy. PLoS One 10: e0130477, 2015.
50. Erickson JR, Pereira L, Wang L, Han G, Ferguson A, Dao K, Copeland RJ, Despa F, Hart GW, Ripplinger CM, *et al*: Diabetic hyperglycaemia activates CaMKII and arrhythmias by O-linked glycosylation. Nature 502: 372-376, 2013.
51. Stølen TO, Høydal MA, Kemi OJ, Catalucci D, Ceci M, Aasum E, Larsen T, Rolim N, Condorelli G, Smith GL, *et al*: Interval training normalizes cardiomyocyte function, diastolic Ca^{2+} control, and SR Ca^{2+} release synchronicity in a mouse model of diabetic cardiomyopathy. Circ Res 105: 527-536, 2009.
52. Bennett CE, Johnsen VL, Shearer J and Belke DD: Exercise training mitigates aberrant cardiac protein O-GlcNAcylation in streptozotocin-induced diabetic mice. Life Sci 92: 657-663, 2013.