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An Invited Review following *the Soujinkai Young Investigator Award*: Modulating Calmodulin-Ryanodine Receptor Binding as a Strategy to Halt Pressure-Overload Induced Cardiac Hypertrophy

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Abstract Cardiac hypertrophy is widely recognized as a significant risk factor contributing to adverse outcomes in individuals with cardiovascular conditions. The disruption of intracellular calcium (Ca^{2+}) balance has been implicated in the development of cardiac hypertrophy, though the precise mechanisms remain poorly understood. In this research, we explored whether hypertrophy induced by pressure overload may arise from the destabilization of the cardiac ryanodine receptor (RyR2) triggered by the dissociation of calmodulin (CaM), leading to subsequent Ca^{2+} leakage. We also assessed whether genetically strengthening the binding affinity between CaM and RyR2 could potentially reverse this process. In the early phases of cardiac hypertrophy caused by pressure overload—when contractile function is still intact—we observed that RyR2 destabilization mediated by reactive oxygen species (ROS) coincides with impaired relaxation. Moreover, stabilizing RyR2 through enhanced CaM binding was found to completely inhibit hypertrophic signaling and improve survival rates. Our findings reveal a crucial connection between RyR2 destabilization and the progression of cardiac hypertrophy.

Key words: calmodulin, ryanodine receptor, cardiac hypertrophy

Introduction

Cardiac hypertrophy is recognized as a significant risk factor for adverse outcomes in patients with cardiovascular diseases. At first, pressure overload causes hypertrophy, which is generally viewed as a compensatory mechanism to balance wall stress. However, when hypertrophy persists, it becomes harmful, leading to elevated oxygen consumption and increased cardiomyocyte loss.[1]

Alterations in Ca^{2+} handling, such as Ca^{2+} leakage through RyR2 influence hypertrophic signaling and electrical remodeling,[2,3]

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and pharmacological inhibition of Ca^{2+} leakage led to attenuation of cardiac hypertrophy.[3] The exact mechanisms through which abnormal diastolic Ca^{2+} leakage triggers signals for cardiac hypertrophy, and the ways in which inhibiting this leakage can suppress hypertrophy, are not yet fully understood. In this study, we examined whether the RyR2 mutation V3599K, which enhances calmodulin (CaM) binding affinity to RyR2[4] to reduce diastolic Ca^{2+} leakage, could prevent the progression of hypertrophy due to pressure overload and avert subsequent heart failure.[5] Our findings suggest that genetically preventing CaM dissociation from RyR2 effectively reduces Ca^{2+} leakage, thereby alleviating cardiac hypertrophy and enhancing clinical outcomes.[5] In this review, we present our proposed approach of stabilizing the RyR2 tetramer as a therapeutic strategy to combat hypertrophy resulting from pressure overload and to prevent subsequent heart failure.

Zipping/Unzipping hypothesis

Building on the domain-switch hypothesis introduced by Ikemoto and colleagues, our research has shown that impaired inter-domain interactions between the N-terminal domain (amino acids 1-220) and the central domain (amino acids 2250-2500) of RyR2, a process known as domain unzipping, leads to Ca^{2+} leakage through RyR2. This leakage contributes to catecholaminergic polymorphic ventricular tachycardia (CPVT) and heart failure.[4,5] These findings strongly suggest that in heart failure pathogenesis, domain unzipping is allosterically connected to conformational shifts in the CaM binding domain (amino acids 3583-3603), leading to CaM dissociation and subsequent Ca^{2+} leakage.

While the zipping-unzipping hypothesis provides a broad explanation for the pathogenic mechanism of Ca^{2+} leakage in CPVT and heart failure, high-resolution structures obtained through cryo-electron microscopy (cryo-EM) have shown that CPVT-causing mutations do not affect a single interface. Instead, they impact multiple smaller interfaces between domains, both within and across critical hotspots.[11,12] (Fig. 1)

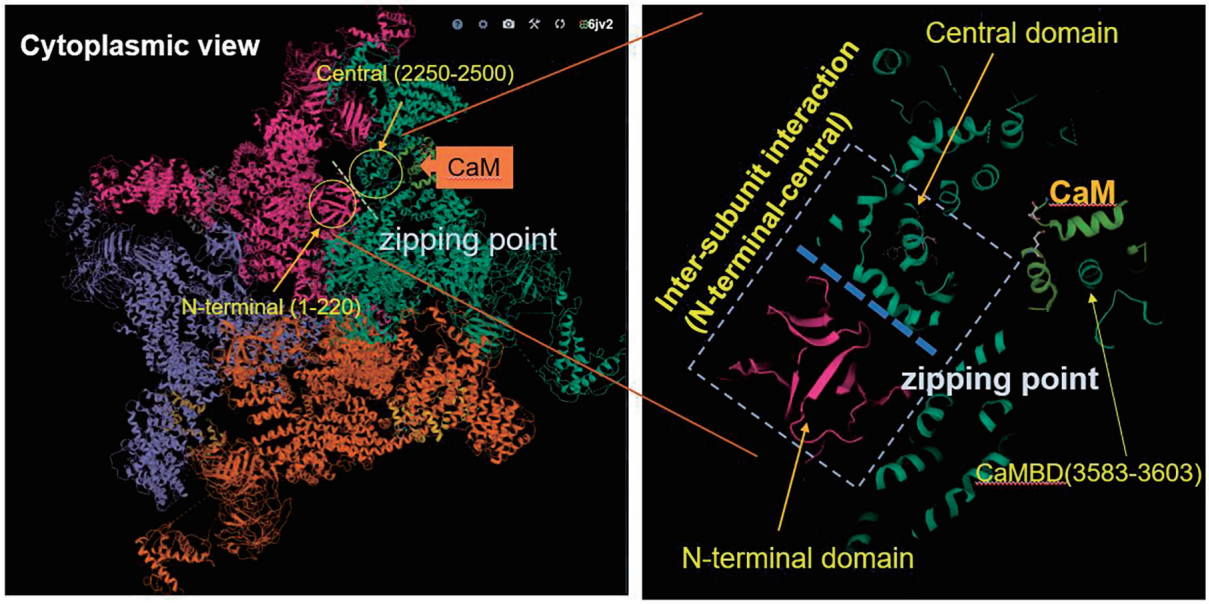

Fig 1. Structural mechanism by which CaM binding stabilizes the RyR tetramer (based on the three-dimensional structure from PDB ID: 6JV2, ref. [12]).

The interface between the N-terminal (amino acids 1-220; red) and central (amino acids 2250-2500; green) domains forms an inter-subunit interaction. The calmodulin (CaM)-binding domain (amino acids 3583-3603) is located near this inter-subunit interface.

RyR2 V3599K mutation suppressed the development of cardiac hypertrophy

Before transverse aortic constriction (TAC), there were no significant differences in cardiac structure or function between wild-type (WT) and V3599K mice. After TAC, WT mice exhibited increased left ventricular (LV) wall thickness and LV weight within two weeks, whereas V3599K mice showed no such changes. At eight weeks post-TAC, WT mice had pronounced LV chamber enlargement due to hypertrophy, along with interstitial fibrosis in cardiomyocytes, which was absent in V3599K cardiomyocytes. These structural and functional changes were notably reduced in V3599K mice, which also showed significantly higher survival rates compared to WT mice.

RyR2 V3599K mutation suppressed dissociation of CaM from RyR2 and diastolic Ca^{2+} leak

In WT cardiomyocytes, the frequency of Ca^{2+} sparks increased even two weeks after TAC, potentially leading to prolonged Ca^{2+} transients. However, these abnormalities were restored in V3599K cardiomyocytes. The Ca^{2+} content of the sarcoplasmic reticulum (SR) decreased eight weeks after TAC in WT cardiomyocytes but remained unchanged in V3599K cardiomyocytes.

Endogenous CaM was co-localized with RyR2 on the Z-line in WT cardiomyocytes prior to TAC. In contrast, it was displaced after TAC in WT cardiomyocytes. In V3599K cardiomyocytes, endogenous CaM remained normally associated with RyR2 along the Z-line. There was no difference in endogenous CaM levels between the control group (before TAC) and the V3599K group.

Effect of enhancement CaM binding affinity to RyR in the development of cardiac hypertrophy

To investigate the exact mechanisms of hypertrophic signaling following pressure overload, we developed a chamber system that can apply air-compressive pressure overload to cardiomyocytes at any point after electrical pacing. Our experiments revealed novel findings: abnormal interactions between calmodulin and RyR2 play a crucial role in initiating pressure-overload hypertrophy. Supporting this hypothesis, our whole transcriptome analysis showed that hypertrophy-related genes, such as Acta1, Myh7, Nppa, and Nppb, did not increase after transverse aortic constriction when CaM dissociation was genetically prevented. There are two major Ca^{2+}-activated cardiac hypertrophy signaling pathways, the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)- HDAC pathway and the CnA- NFAT pathway.[13,14] Phosphorylation of HDAC by CaMKII causes HDAC to be exported from the nucleus, thereby lifting HDAC-mediated transcriptional repression on MEF2 and promoting MEF2-dependent transcription of hypertrophic genes. Additionally, CnA dephosphorylates NFAT, facilitating NFAT's nuclear translocation and enhancing NFAT-dependent transcription of hypertrophic genes, such as ANP and GATA4.[14] Consistent with previous research, pathway analysis in the TAC model identified MEF2C as an upstream regulator, with its activation occurring through GATA4 activation and HDAC inhibition.

Notably, our findings revealed that pressure-overload-induced RyR2 destabilization supplies both critical factors required for two primary hypertrophic signaling pathways: CaM and an increase in diastolic Ca^{2+}. Furthermore, we discovered that genetically restoring CaM binding to RyR2 successfully inhibited the translocation of HDAC and NFAT (Fig. 2).

Clinical perspectives

This study showed that RyR2-V3599K mutation prevented diastolic Ca^{2+} leak through RyR2 via enhancing the binding affinity of calmodulin (CaM) to RyR2, resulting in inhibition of cardiac hypertrophy and heart failure.[5] Therefore, genetically stabilizing the tetrameric structure of RyR2 improved survival rates in cases of pressure-overloaded left ventricular hypertrophy. This effect is achieved by strengthening CaM's binding affinity to RyR2, which in turn inhibits diastolic Ca^{2+} leakage through RyR2. These findings lay the groundwork for clinical studies,

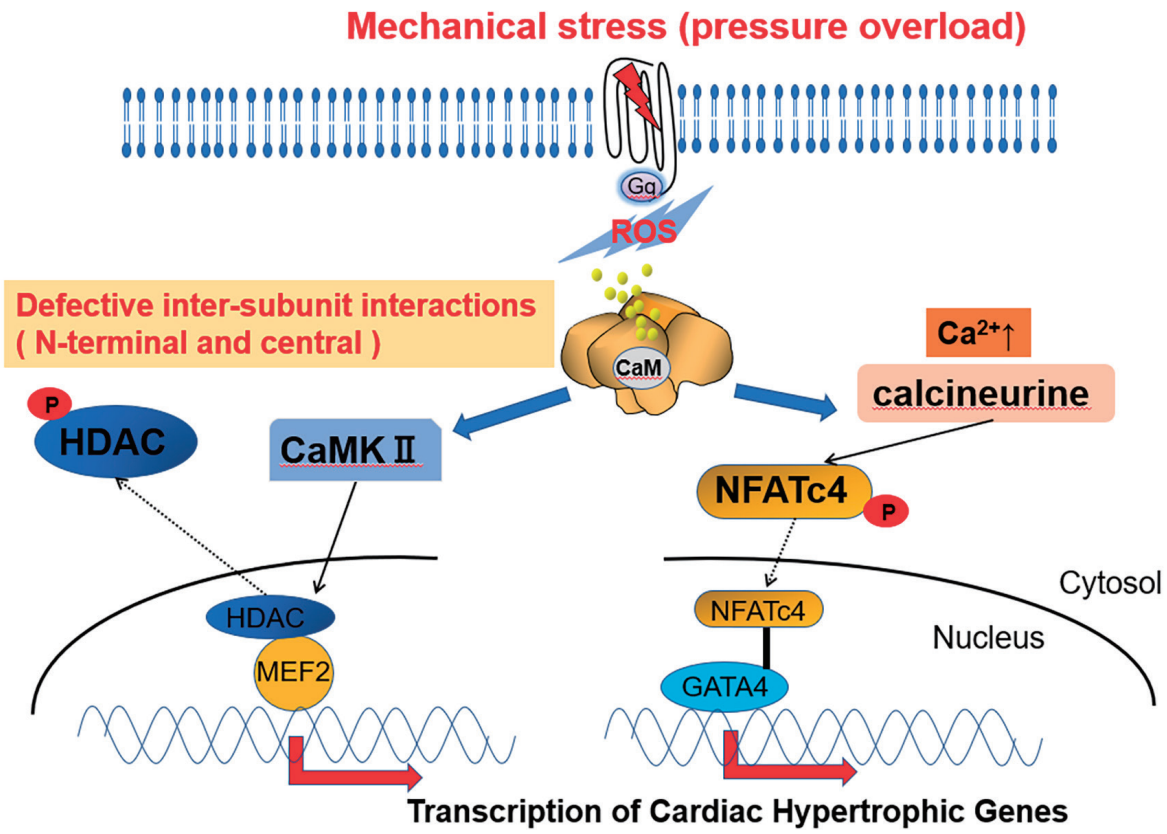

Fig 2. Pressure overload induces cardiac hypertrophy.
Mechanical stress leads to ROS production, causing CaM dissociation and Ca^{2+} leakage, which in turn activates hypertrophic signaling via the CaM-CaMKII and Ca^{2+}-calcineurin pathways.

with potential applications that could enhance prognosis for patients with pressure-overloaded heart failure.

Conflict of interest

The authors declare no conflict of interest.

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