HHS Public Access

Author manuscript

J Mol Cell Cardiol. Author manuscript; available in PMC 2021 December 01.

Published in final edited form as:

J Mol Cell Cardiol. 2020 December ; 149: 73–81. doi:10.1016/j.yjmcc.2020.09.007.

CaMKII exacerbates heart failure progression by activating class I HDACs

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Abstract

Background: Persistent cardiac Ca^{2+}/calmodulin dependent Kinase II (CaMKII) activation plays an essential role in heart failure development. However, the molecular mechanisms underlying CaMKII induced heart failure progression remains incompletely understood. Histone deacetylases (HDACs) are critical for transcriptional responses to stress, and contribute to expression of pathological genes causing adverse ventricular remodeling. Class I HDACs, including HDAC1, HDAC2 and HDAC3, promote pathological cardiac hypertrophy, whereas class IIa HDACs suppress cardiac hypertrophy. While it is known that CaMKII deactivates class IIa HDACs to enhance cardiac hypertrophy, the role of CaMKII in regulating class I HDACs during heart failure progression is unclear.

Methods and Results: CaMKII increases the deacetylase activity of recombinant HDAC1, HDAC2 and HDAC3 via *in vitro* phosphorylation assays. Phosphorylation sites on HDAC1 and HDAC3 are identified with mass spectrometry. HDAC1 activity is also increased in cardiac-specific CaMKIIδ_C transgenic mice (CaMKIIδ_C-tg). Beyond post-translational modifications,

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AUTHOR CONTRIBUTIONS

M.Z and N.F designed and conceived the experiments. M.Z, R.Z, X.Y, Q.W, M.A.R, J.M.G, E.D.L, D.B and N.F performed experiments and analyzed data. H.J analyzed the data and interpreted the results. M.Z and N.F wrote and edited the manuscript.

CONFLICT OF INTEREST

None to report.

CaMKII induces HDAC1 and HDAC3 expression. HDAC1 and HDAC3 expression are significantly increased in CaMKIIδ_C-tg mice. Inhibition of CaMKII by overexpression of the inhibitory peptide AC3-I in the heart attenuates the upregulation of HDAC1 after myocardial infarction surgery. Importantly, a potent HDAC1 inhibitor Quisinostat improves downregulated autophagy genes and cardiac dysfunction in CaMKIIδ_C-tg mice. In addition to Quisinostat, selective class I HDACs inhibitors, Apicidin and Entinostat, HDAC3 specific inhibitor RGFP966, as well as HDAC1 and HDAC3 siRNA prevent CaMKII overexpression induced cardiac myocyte hypertrophy.

Conclusion: CaMKII activates class I HDACs in heart failure, which may be a central mechanism for heart failure progression. Selective class I HDACs inhibition may be a novel therapeutic avenue to alleviate CaMKII hyperactivity induced cardiac dysfunction.

Keywords

Ca^{2+}/calmodulin dependent Kinase II; Histone deacetylase; heart failure

1. INTRODUCTION

Heart failure is one of the leading causes of death worldwide and represents a major healthcare burden[1]. Novel mechanism based therapies for heart failure are in high demand. Neurohormonal hyperactivity including persistent activation of β-adrenergic and angiotensin II (AngII) signaling is one of the fundamental mechanisms of adverse ventricular remodeling and heart failure development. As such, neurohormonal inhibition is the cornerstone of current heart failure therapy [1]. Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is a direct downstream effector of β-adrenergic[2] and AngII signaling[3], promoting cardiac hypertrophy[4–6], oxidative stress[3, 7], cell death[8, 9], arrhythmia[10], inflammation[11] and fibrosis[12]. Importantly, CaMKII activity is persistently elevated in heart failure patients despite standard neurohormonal inhibition therapies [13]. Therefore, new strategies need to be developed to mitigate the adverse effects of CaMKII hyperactivity.

The molecular mechanisms of CaMKII mediated pathological cardiac hypertrophy remain poorly understood. CaMKII is known to inhibit class IIa histone deacetylases (HDACs)[14, 15], such as HDAC4 and HDAC5. Class IIa HDACs prevent cardiac hypertrophy by the suppression of the pro-hypertrophic transcription factor myocyte enhancer factor-2 (MEF2) [16]. The inhibition of class IIa HDACs by CaMKII results in exacerbated cardiac hypertrophy. In contrast to class IIa HDACs, class I HDACs promote cardiac hypertrophy through a number of mechanisms, including the suppression of inositol polyphosphate-5-phosphatase f (Inpp5f) expression and subsequent inhibition of glycogen synthase kinase 3β (GSK3β) signaling[17], or the inhibition of dual-specificity phosphatase 5 (DUSP5), a nuclear phosphatase that negatively regulates ERK1/2 elicited cardiac hypertrophy[18], or by attenuating autophagy via activation of mTOR signaling[19]. Here we investigated whether CaMKII regulates class I HDACs and whether class I HDAC inhibitors, many of which are already in clinical use[20], could represent novel therapies to antagonize persistently elevated CaMKII activity in heart failure patients.

2. METHODS

2.1 Animal models and procedures

Study procedures were approved by the Johns Hopkins University and University of Pittsburgh Animal Care and Use Committees in accordance with National Institutes of Health guidelines. Cardiac-specific CaMKIIδ_C transgenic mice (CaMKIIδ_C-tg)[21] and cardiac-specific transgenic mice overexpressing CaMKII inhibitory peptide (AC3-I)[4] were generated as reported previously. CaMKIIδ_C-tg mice with 17 fold increase of the amount of CaMKII rapidly progress to heart failure and premature death. At the age of 8 weeks, CaMKIIδ_C-tg mice display 50% increases in left ventricular mass to body weight ratio, 35% to 45% increases in Left ventricular end diastolic diameter (LVEDD), and 50% to 60% decreases in fractional shortening, compared to WT controls. [21] AC3-I and control mice were subjected to myocardial infarction by LAD ligation as described previously[22].Briefly, AC3-I mice and controls were induced with 3–4% isoflurane for 2–3 minutes and intubated with 20g Jelco IV catheter. The mice were mechanically ventilate at 200μl tidal volume and 120bpm, and the sedation was maintained with isoflurane to 1.5–2.5%. After the mice were paralyzed with succinylcholine, left thoracotomy in the 5th to 6th intercostal space was performed. Proximal LAD was permanently ligated using 7–0 prolene and the myocardial infarction was verified with the blanching of the tissue distal to the suture. After LAD ligation, the chest was closed and the mice were extubated after they regained respiration. Hearts were harvested 7 days post-infarction.

CaMKIIδ_C-tg mice at the age of 6–8 weeks were randomly assigned to vehicle or Quisinostat treatment group (10mg/kg/day, dissolved in 5% DMSO in saline, either delivered by IP for three days or by Alzet pump for 2 weeks). We chose Quinsinostat because it is the most potent and relatively selective HDAC1 inhibitor. The IC50 of Quisinostat to HDAC1 is 0.11nM in a cell-free assay, whereas the IC50 to HDAC4 is 0.64nM, 6-fold difference. Quisinostat has greater than 30-fold selectivity against other class II HDACs including HDAC5, HDAC6, HDAC7 and HDAC9 [23]. The effectiveness of HDAC inhibition by Quisinostat *in vivo* was confirmed with increased histone acetylation (Supplement Fig S4). Serial echocardiography were performed in conscious mice (Acuson Sequoia C256, 13-MHz; Siemens) as described previously[24].

2.2 Cardiac myocytes studies

Neonatal rat cardiac myocytes (NRCMs) were freshly isolated as described previously[25]. Briefly, the hearts were quickly removed from one to three days old Sprague Dawley neonates and immersed into chilled dissociation buffer (pH 7.5) containing: NaCl 137mM, KC1 5.36mM, MgSO4–7H2O 0.81mM, dextrose 5.55mM, KH2PO4 0.44mM, Na2HPO4–7H2O 0.34mM, and HEPES 20mM. The ventricles were cut into 1–2mm pieces and the cardiac myocytes isolation was achieved by digestion with 0.04% trypsin and collagenase 0.4mg/ml in dissociation buffer at 37°C. The digestion was terminated by DMEM containing 10% fetal bovine serum (FBS). Non-cardiomyocyte cells were removed by rapid attachment (90 minutes incubation in culture dishes). Cardiomyocytes were plated at the density of 2×10^5/ml in DMEM containing 10%FBS and 0.1mM BrdU to prevent the growth

of fibroblasts. 24 hours after cells plating, the medium was changed to serum free DMEM containing 0.1% Insulin-transferrin-selenium (Thermo Fisher Scientifics).

NRCM were infected with adenovirus containing the full length human CaMKIIδ_C coding sequence or control virus for 48 hours. The adenoviral CaMKIIδ_C was generated by the Gene Transfer Vector Core at the University of Iowa as described previously[3]. The overexpression of CaMKII was confirmed (Supplement Fig S2). In addition to Quisinostat (10nM, Selleckchem, # S1096), other selective class I HDAC inhibitor Apicidin (0.5μM, Sigma # A8851)[19], Entinostat (25μM, Selleckchem # S1053)[26],or HDAC3 selective inhibitor RGFP966 (10μM, Selleckchem, # S7229)[27] were added 24 hours after CaMKIIδ_C viral infection for cardiomyocytes hypertrophy studies. In some studies, NRCM were added with phenylephrine (20μM, Sigma), CaMKII inhibitor KN93 or KN92 (KN93 inactive analog) (5μM, Sigma), JNK inhibitor SP600125 (20μM, Sigma), or AP1 inhibitor SR11302 (10μM, Tocris Bioscience) for 48 hours. Cardiomyocytes hypertrophy was assessed with cell surface area in addition to ANP/BNP mRNA level. Immunostaining of sarcomeric α-actinin was performed (Abcam #ab9465) and the cell surface area was measured with ImageJ.

To further demonstrate that class I HDACs are the downstream targets of CaMKII, HDAC1 and HDAC3 siRNA (Origene #SR505395, #SR505399) were used in the cardiomyocytes hypertrophy studies. NRCMs were transfected with HDACs siRNA using Lipofectamine™ RNAiMAX Transfection Reagent (Thermo Fisher Scientifics) according to Manufacture's protocol. The knockdown of HDACs was confirmed (supplement Fig S3). The specificity of the HDAC1 and HDAC3 antibody (Cell Signaling Technologies Cat#5356 and #3949 respectively) was also validated in the knockdown experiments. The Phenylephrine was added 24 hours after siRNA transfection.

2.3 *In vitro* histone deacetylase activity assay

HDAC activity was measured using a fluorescent assay kit (Active Motif, Cat# 56200) according to the manufacturer's instruction. Recombinant HDAC1 and HDAC3 proteins were purchased from Enzo Life Sciences (cat# BML-SE456–0050, BML-SE507–0050). Recombinant CaMKII protein was generated using the Bac-to-Bac baculovirus system (Invitrogen) and purified on a calmodulin-agarose column as described previously [3]. *In vitro* phosphorylation of HDAC1 or HDAC3 was carried out by incubation of recombinant HDAC1 or HDAC3 protein (125ng) with CaMKII (125, 250, 500ng) in buffer mixture containing 50mM HEPES, MgAC2 10mM, CaCl2 0.5mM, calmodulin 1μM, and ATP 0.4mM, at 30°C for 1 hour. CaMKII alone, HDAC inhibitor Trichostatin A (TSA) were used as negative controls.

Mass spectrometry to evaluate the phosphorylation sites of HDACs by CaMKII was performed at the Mass Spectrometry and Proteomics Core of Johns Hopkins University and the Biomedical Mass Spectrometry Center of University of Pittsburgh.

2.4 Western blot, coimmunoprecipitation

Protein extracts were prepared in RIPA lysis buffer (Thermo Fisher Scientifics) from snap-frozen heart tissues or NRCMs. Protein concentration was measured by BCA assay (Thermo

Fisher Scientifics). The protein electrophoresis were performed on 4–12% Bis-Tris NuPage gels (Thermo Fisher Scientifics). The Bio-Rad Trans-Blot Turbo Transfer System was used for the proteins transfer to nitrocellulose membranes. Secondary antibodies used were from LI-COR Biosciences. The blots were quantified using Image J or LI-COR Image studio Lite. HDAC1, HDAC2 and HDAC3 antibodies were from Cell Signaling Technologies (Cat#5356, #5113 and #3949 respectively). The Sin3a antibody was from Abcam, # ab129087.

Co-immunoprecipitation: HDAC1 was immunoprecipitated from 500μg heart lysates (in RIPA buffer) using 0.5μg HDAC1 antibody (Cell Signaling Technologies Cat#5356), and 25 μl Dynabeads™ Protein G (Thermo Fisher Scientifics). Co-immunoprecipitated HDAC2 and Sin3a were probed using antibody from Cell Signaling Technologies (#5113) and Abcam (# ab129087) respectively.

2.5 Quantitative RT-PCR

Total RNA was extracted from either NRCMs or snap-frozen heart tissues using TRIzol reagent (Thermo Fisher Scientifics). Reverse transcription was conducted using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientifics). Taqman primers (Thermo Fisher Scientifics) were used for quantitative RT-PCR analysis: rat NPPA (Rn00664637_g1) and NPPB (Rn00580641_m1), mouse Atg2a (Mm01212087_m1), mouse Atg4b (Mm03031857_s1), mouse Atg12 (Mm00503201_m1), mouse GABARAPL1 (Mm00457880_m1), mouse ULK1 (Mm00437238_m1), and mouse TSC2 (Mm00442004_m1).

2.6 Statistics

All values are represented as the mean ± SEM. Data were compared within the groups using one-way or two-way ANOVA with Tukey's Post Hoc Test using GraphPad Prism version 6.0. Unpaired student's *t* test was used for comparison between two groups. Paired student's *t* test was used for before and after treatment of Quisinostat in CaMKIIδ_C-tg mice. All tests were two-tailed and a P value of less than 0.05 was considered significant.

3. RESULTS

3.1 CaMKII directly enhances HDAC1 activity by phosphorylation

CaMKII regulates HDAC4 signaling, a class IIa HDAC, through phosphorylation. CaMKII mediated phosphorylation of HDAC4 initiates translocation of HDAC4 from the nucleus into the cytoplasm by binding to 14-3-3 protein[14]. Class I HDACs are predominantly located in nucleus. Class I HDACs possess much stronger deacetylase activity than class IIa HDACs[28]. We first examined whether CaMKII could modulate class I HDACs deacetylase activity through phosphorylation. Recombinant HDAC1, HDAC2 and HDAC3 were incubated with CaMKIIδ protein, Ca^{2+}/calmodulin and ATP. The deacetylase activity of HDAC1 was increased upon CaMKIIδ incubation in a dose-dependent manner. (Fig 1A). Similarly, HDAC2 and HDAC3 deacetylase activity were markedly increased with CaMKIIδ incubation. The baseline deacetylase activity and CaMKII-induced deacetylase enhancement were completely blocked by Trichostatin A (TSA). CaMKII did not have deacetylase

activity (Fig 1A). Without Ca^{2+}/calmodulin and ATP, We found that CaMKII alone lost the ability to enhance HDAC1 activity, suggesting that phosphorylation is required for CaMKII action (Fig 1A). The phosphorylation sites of HDACs by CaMKII was evaluated by mass spectrometry after incubation. Two independent experiments revealed CaMKII phosphorylated HDAC1 at T65, S69, S85, T195, S197, and T355 and HDAC3 at S374 (Supplement Fig S1). Next we tested whether HDAC1 activity is increased in cardiac-specific CaMKII transgenic mice ($CaMKII\delta_C$-tg). HDAC1 was immunoprecipitated from WT control and $CaMKII\delta_C$-tg mice hearts and HDAC1 activity was measured and normalized by the amount of HDAC1. Consistent with *in vitro* study, HDAC1 activity in $CaMKII\delta_C$-tg mice was significantly elevated relative to WT controls. (Fig 1B).

3.2 CaMKII increases HDAC1 and HDAC3 expression *in vitro* and *in vivo*

To determine whether CaMKII regulates class I HDACs signaling beyond post-translational level, we investigated the effect of CaMKII on HDAC1 and HDAC3 expression. In cultured neonatal rat cardiac myocytes (NRCMs) overexpressing CaMKIIδc with adenoviral transduction, HDAC1 and HDAC3 expression were markedly increased (Fig 2A). The overexpression of CaMKIIδc was confirmed (Supplement Fig S2). In cultured NRCM challenged with phenylephrine (PE, cardiac myocyte hypertrophy inducer), HDAC1 expression was markedly increased. However, the upregulation of HDAC1 was blunted by the incubation of CaMKII inhibitor KN93, but not KN92 (Fig 2B). Mirroring this *in vitro* data, HDAC1 and HDAC3 were significantly increased In $CaMKII\delta_C$-tg mice relative to WT controls (Fig 2C). Furthermore, while HDAC1 expression was significantly elevated in WT control mice subjected to myocardial infarction, this was attenuated in cardiac-specific transgenic mice overexpressing CaMKII inhibitory peptide AC3-I (Fig 2D). This highlights the critical role of CaMKII in regulating of class I HDACs expression in the stressed heart. We next investigated the potential mechanisms of the HDAC1 level elevation by CaMKII overexpression. We found that the C-Jun N-Terminal Kinase (JNK)/ AP-1 transcription factor pathway was implicated in the CaMKII mediated HDAC1 upregulation, as JNK inhibitor SP600125 or AP-1 inhibitor SR11302 blunted the elevation induced by CaMKII overexpression or PE (Fig 2E). Taken together, our findings suggests CaMKII regulates class I HDACs signaling on both transcriptional and post-translational level.

3.3 CaMKII enhances HDAC1/HDAC2 repressive complex formation

HDAC1 and HDAC2 often bind together and recruit other transcriptional repressors including Sin3a to form multi-protein repressive complexes. We investigated whether the HDAC1/2/Sin3a complex formation are enhanced in CaMKIIδc-tg mice by co-immunoprecipitation (Co-IP) of HDAC1 from heart lysates. To demonstrate the increased HDAC1/HDAC2/Sin3a repressive complex formation is due to CaMKII-induced binding affinity enhancement, rather than increased expression level of HDAC1, we loaded the same level of immunoprecipitated HDAC1. For the normalized immunoprecipitated HDAC1, the co-immunoprecipitated HDAC2 and Sin3a were increased in CaMKIIδc-tg mice. These findings suggested CaMKII directly promotes HDAC1/HDAC2/Sin3a formation. (Fig 3).

3.4 Class I HDACs inhibition attenuates CaMKII overexpression induced cardiac myocyte hypertrophy

To determine if the CaMKII-class I HDACs signaling pathway indeed plays a critical role in cardiac hypertrophy, we tested whether class I HDACs inhibition would ameliorate persistent CaMKII activation induced cardiac myocyte hypertrophy. CaMKII overexpression in NRCMs resulted in hypertrophy, as assessed by ANP/BNP mRNA expression and cell surface area (Fig 4A, B). Quisinostat, the most potent HDAC1 inhibitor attenuated CaMKII-induced hypertrophy (Fig 4A, B). In addition to Quisinostat, class I HDAC selective inhibitors Apicidin and Entinostat also attenuated CaMKII-induced hypertrophy. RGFP966, a HDAC3 specific inhibitor, blunted CaMKII-induced cardiomyocyte hypertrophy (Fig 4A). In consistent with the HDAC inhibitors, siRNA against HDAC1 and HDAC3 mitigated CaMKII-induced hypertrophy (Fig 4C). Collectively, these findings suggest class I HDACs are one of CaMKII downstream targets promoting cardiac hypertrophy.

3.5 HDAC1 inhibitor Quisinostat improves cardiac dysfunction in CaMKIIδ_C-tg mice

CaMKIIδc-tg mice develop heart failure rapidly and usually die 3–5 months after birth. In agreement with the *in vitro* studies, Quisinostat administration for 14 days (Alzet pump, 10mg/kg/day) in CaMKIIδ_C-tg mice at the age of 6–8 weeks significantly slowed down the progression of CaMKII induced cardiac dysfunction (Fig 5, Δfractional shortening in vehicle vs Quisinostat, −10±2.248% (from 20.5±2.3% to 10.2±1.1%) vs −2.523±1.901 (from 20.7±2.8 to 18.2±2.5%), Vehicle n=6, Quisinostat n=7, P=0.022). Therefore, HDAC1 inhibition indeed improves CaMKII hyperactivity induced cardiac dysfunction. Quisinostat has 6-fold selectivity against HDAC1 than HDAC4, and more than 30-fold selectivity against other class II HDACs. It is unclear whether class IIa HDACs were inhibited in vivo at the dose of 10mg/kg/day. However, because class IIa HDACs are protective in heart failure, and they are direct targets of CaMKII and would be inhibited by the overexpression of CaMKII, the resultant effect of Quisinostat on class IIa HDACs would be negligible or even offset the effect of Quisinostat on class I HDACs. As such, the improvement of cardiac function in CaMKIIδc transgenic mice with Quisinostat is most likely through the inhibition of class I HDACs.

3.6 HDAC1 inhibition restores CaMKII elicited autophagy genes downregulation

We next explored the downstream targets of CaMKII/HDAC1 regulatory pathway. It has been reported that HDAC inhibitors improve impaired autophagy in cardiac hypertrophy by restoring downregulated Tuberous Sclerosis Complex Subunit 2 (TSC2). Here we examined whether autophagy genes are one of the targets of CaMKII/HDAC1.We found, TSC2, Autophagy Related 2A (ATG2A), ATG4B, ATG12, Unc-51 Like Autophagy Activating Kinase 1 (ULK1) and GABA Type A Receptor Associated Protein Like 1 (GABARAPL1) were downregulated in CaMKIIδ_C-tg mice. Administration of Quisinostat (10mg/kg/day, IP) for 3 days restored the expression of these autophagy related genes toward WT levels in CaMKIIδ_C-tg mice (Fig 6).

4. DISCUSSION

Persistent CaMKII activation, either by canonical Ca^{2+}/calmodulin activation or non-canonical oxidative activation[3], plays an essential role in pathological cardiac hypertrophy and adverse ventricular remodeling[4–6]. CaMKII is a direct downstream target of β-adrenergic signaling[2] and Gαq signaling[3] (Endothelin, Angiotensin II), and mediates neurohormonal hyperactivity driven cardiac myocyte death[8], cardiac hypertrophy[4–6], Ca^{2+} mishandling[29], and fibrosis[12]. A recent study shows CaMKII activity remains elevated in heart failure patients despite adequate standard neurohormonal inhibition therapy[13]. While the development of a clinically applicable CaMKII inhibitor is uncertain, an alternative strategy is the pharmacological modulation of CaMKII downstream targets. In this study, we have shown that CaMKII promotes class I HDACs signaling at multiple levels of regulation. CaMKII directly phosphorylates HDAC1, HDAC2 and HDAC3, and enhances their deacetylase activity in *in vitro* assay. Among the identified candidate sites in HDAC1 identified by Mass Spectrometry, the T195 site correlates well with CaMKII kinase consensus sequence XRXXS/TX. In the present study, the phosphorylation sites identified by mass spectrometry might be incomplete, as there was less than 50% coverage of the HDAC amino acid sequences, which were mostly located at the N-terminus. Additional studies will be required to uncover the full spectrum of phosphorylation sites and determine whether these phosphorylation sites exist and have functional relevance in vivo. In addition to this post-translational regulation, CaMKII also induces HDAC1 and HDAC3 expression. Moreover, CaMKII promotes HDAC1 and Sin3A machinery complex formation. Class I HDAC inhibitors have been used clinically for cancer therapy[20] and the inhibition of class I HDAC has been shown to blunt cardiac hypertrophy[30]. Our findings not only reveal one of the mechanisms underlying the protective effect of HDAC inhibitors, but also suggest a strategy to mitigate the adverse consequences of the elevated CaMKII activity in heart failure patients. Indeed, we have shown that class I HDAC inhibitors or non-selective HDAC inhibitor Quisinostat attenuates CaMKII overexpression induced cardiac hypertrophy and dysfunction *in vitro* and *in vivo*.

CaMKIIδ is the predominant cardiac isoform. There are two major subtypes of CaMKIIδ. $CaMKII\delta_B$ containing an 11-amino acid nuclear localization sequence, is thought mainly located in nucleus, while $CaMKII\delta_C$ is mainly present in cytosol[31]. Subsequent studies suggest the subcellular location of CaMKIIδ subtypes is not exclusive, and $CaMKII\delta_C$ is found to play a critical role in transcription regulation[31]. It is reported that $CaMKII\delta_C$ overexpressed in mouse heart increased phosphorylation of HDAC4, resulting in the activation of the transcription factor MEF2[32]. Our findings add the support that $CaMKII\delta_C$ is an essential regulator of gene transcription.

Among the HDAC super family, class I (HDAC1, 2, 3, and 8) and class IIa (HDAC4, 5, 7 and 9) HDACs have been relatively more extensively studied in cardiac hypertrophy and heart failure[33]. Class IIa HDACs are considered cardiac protective, because overexpression of HDAC4[14], HDAC5[34] or HDAC9[35] in cardiac myocytes leads to suppression of MEF2 expression (a pro-hypertrophy transcription factor) and attenuation of stress induced cardiac hypertrophy[16]. In contrast, silencing of HDAC5[36] or HDAC9 results in exacerbated hypertrophic response to pressure overload[35]. CaMKII modulates

class IIa HDACs interaction with MEF2 by directly phosphorylating HDAC4[14]. Phosphorylation of HDAC4 prompts its binding to 14-3-3 protein which in turn exports HDAC4 out of the nucleus[14]. Therefore, CaMKII relieves class IIa HDACs suppression on MEF2, and aggravates cardiac hypertrophy and ventricular remodeling. In contrast, Class I HDACs are generally considered as pro-hypertrophy and detrimental in heart failure[33, 37, 38]. Cardiomyocyte-specific overexpression of HDAC2 provokes severe cardiac hypertrophy[17], and HDAC3 overexpression causes hyperplasia[39]. In addition, Class I HDAC specific inhibitors attenuates cardiac hypertrophy[19, 30]. Our findings suggest that CaMKII promotes cardiac hypertrophy and adverse ventricular remodeling by orchestrating two opposing classes of HDACs (Fig 7). CaMKII enhances pro-hypertrophic Class I HDACs and suppresses anti-hypertrophic Class IIa HDACs. Class IIa HDACs have weak deacetylase activity[28], and often function as readers, requiring recruitment of class I HDACs to suppress gene expression. CaMKII acts on class IIa HDACs through exporting them out of the nucleus. For Class I HDACs with strong deacetylase activity, CaMKII directly regulates their activity and expression.

CaMKII is activated either by β-adrenergic and AngII induced Ca^{2+}/calmodulin elevation, or by β-adrenergic and AngII induced oxidative modification in heart failure. Class I HDACs exacerbate heart failure progression whereas class IIa HDACs protect against heart failure. CaMKII promotes class I HDACs signaling and inhibits class IIa HDACs signaling. Therefore, CaMKII is a master regulator of HDACs promoting heart failure progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health grant K08 HL130604, American Heart Association Innovative Project Award #18IPA34170219, UPMC Competitive Research Fund (Dr. Ning Feng), and Samuel and Emma Winters Foundation (Dr. Manling Zhang). We thank the support of Center for Biologic Imaging at University of Pittsburgh, and the confocal microscope was supported by NIH S10OD019973.

We greatly appreciate Dr. Mark Anderson's scientific suggestions and critical review of the manuscript. We are grateful for Dr. Iain Scott's critical review of the manuscript. We thank Michelle Leppo for her work on myocardial infarction mouse model. We also thank Dr. Joan Brown for providing us the CaMKIIδ$_C$-tg mice.

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HIGHLIGHTS

- CaMKII directly enhances HDAC1 and HDAC3 deacetylase activity
- CaMKII induces HDAC1 and HDAC3 expression in the heart
- CaMKII promotes HDAC1/HDAC2/Sin3a repressive complex formation
- Class I HDAC inhibitors improve CaMKII hyperactivity induced cardiac hypertrophy
- HDAC inhibitor recovers CaMKII hyperactivity induced autophagy genes downregulation

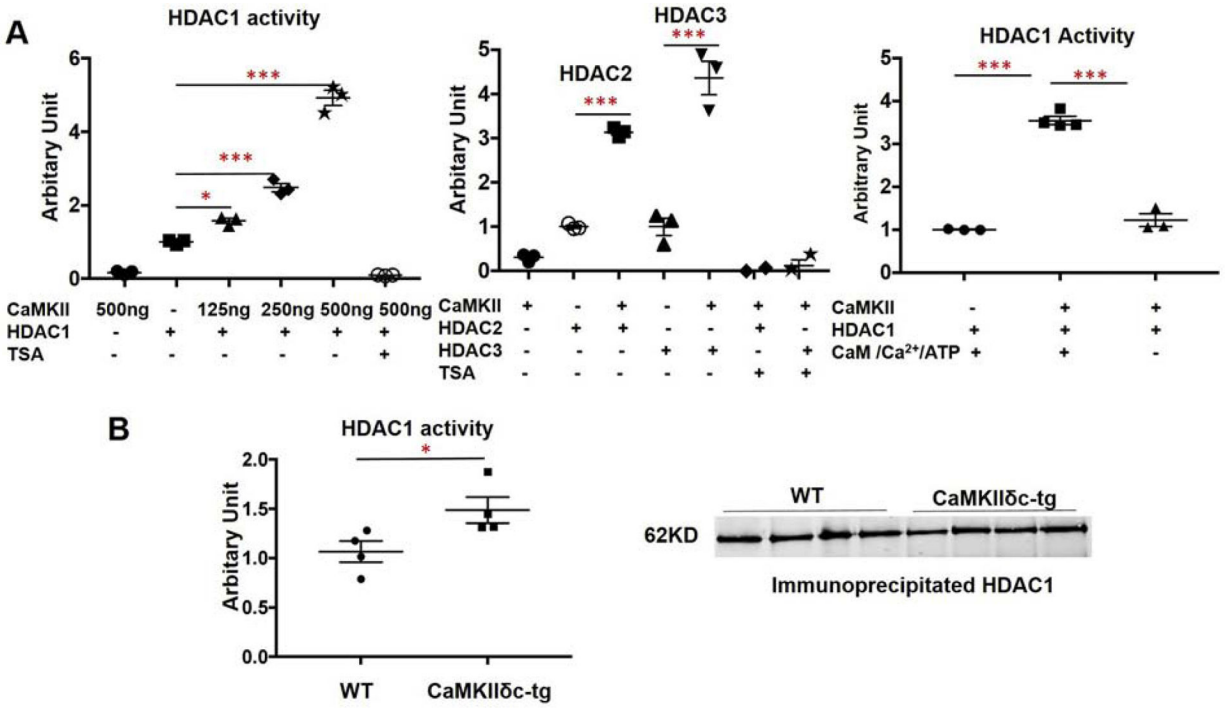

Fig 1. CaMKII enhances class I HDACs activity

A. The deacetylase activity of recombinant HDAC1 was increased with CaMKII incubation in a dose dependent manner. CaMKII enhanced HDAC2 and HDAC3 deacetylase activity (n=3). CaMKII did not have deacetylase activity. CaMKII lost the ability to increase HDAC1 activity without Ca^{2+}/calmodulin(CaM) and ATP. **B.** HDAC1 was immunoprecipitated from WT and CaMKIIδc-tg mice heart lysates. HDAC1 activity was significantly increased to 1.5 fold in CaMKIIδ_C-tg mice hearts (n=4). The activity was normalized by the immunoprecipitated HDAC1 amount. * *P<0.05,* *** *P<0.001*

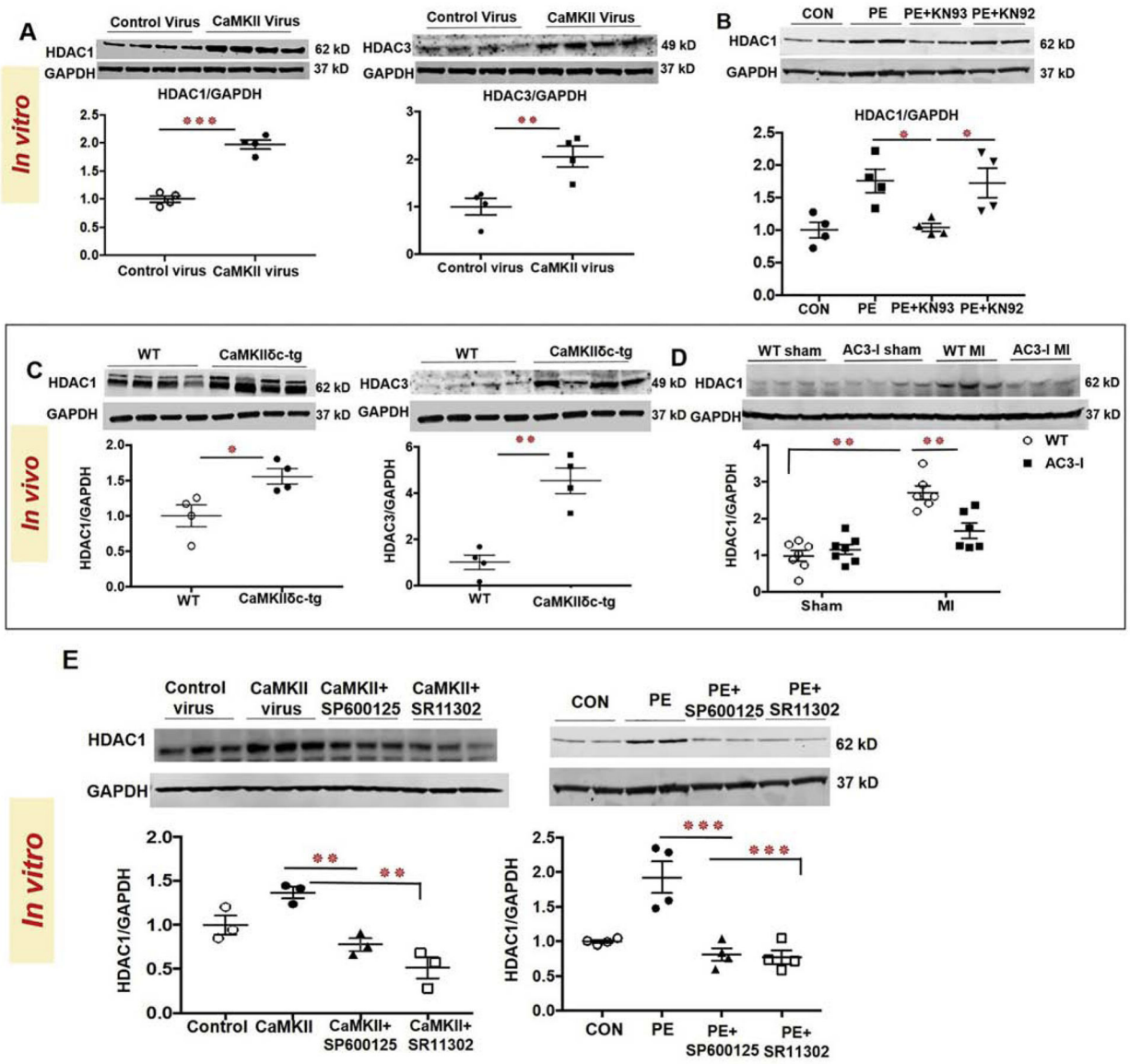

Fig 2. CaMKII upregulates HDAC1 and HDAC3 expression

A. In neonatal rat cardiac myocytes (NRCMs): Overexpression of CaMKII induced HDAC1 and HDAC3 expression (n=4). **B.** Phenylephrine (PE) increased HDAC1 expression and KN93, a CaMKII inhibitor blunted the upregulation. KN92 had no effect on HDAC1 expression (n=4). **C.** HDAC1 and HDAC3 expression were significantly increased in CaMKIIδc-tg mice hearts (n=4). **D.** HDAC1 expression was significantly increased post myocardial infarction (MI), and the upregulation of HDAC1 was attenuated in transgenic mice overexpressing CaMKII inhibitory peptide AC3-I (Sham WT/AC3-I n=7, MI WT/AC3-I n=6). **E.** JNK inhibitor SP600125 or AP-1 inhibitor SR11302 abolished CaMKII (n=3) or PE (n=4) induced HDAC1 elevation, suggesting JNK/AP-1 pathway mediates CaMKII induced HDAC1 upregulation. *$P<0.05$, **$P<0.01$, ***$P<0.001$

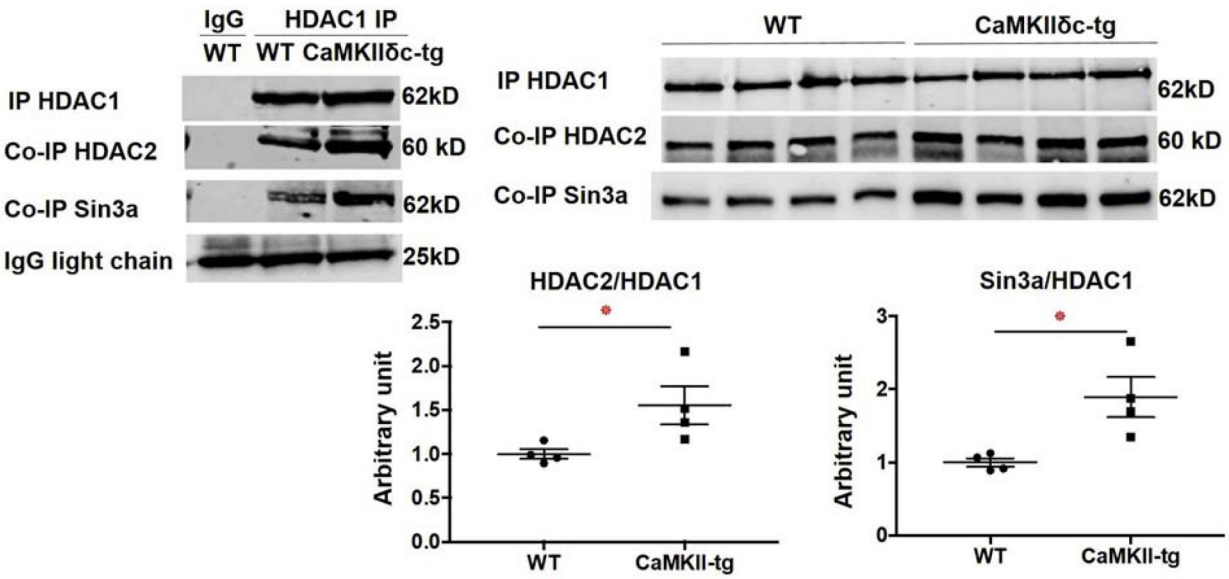

Fig 3. CaMKII promotes HDAC1/HDAC2/Sin3a repressive complex formation

HDAC1 was immunoprecipitated from WT controls and CaMKIIδ_C-tg mice heart lysates. Co-immunoprecipitation showed increased binding of HDAC2 and Sin3a to normalized HDAC1 level in CaMKIIδc-tg mice hearts. Rabbit IgG was used as a negative control. n=4, $*P<0.05$.

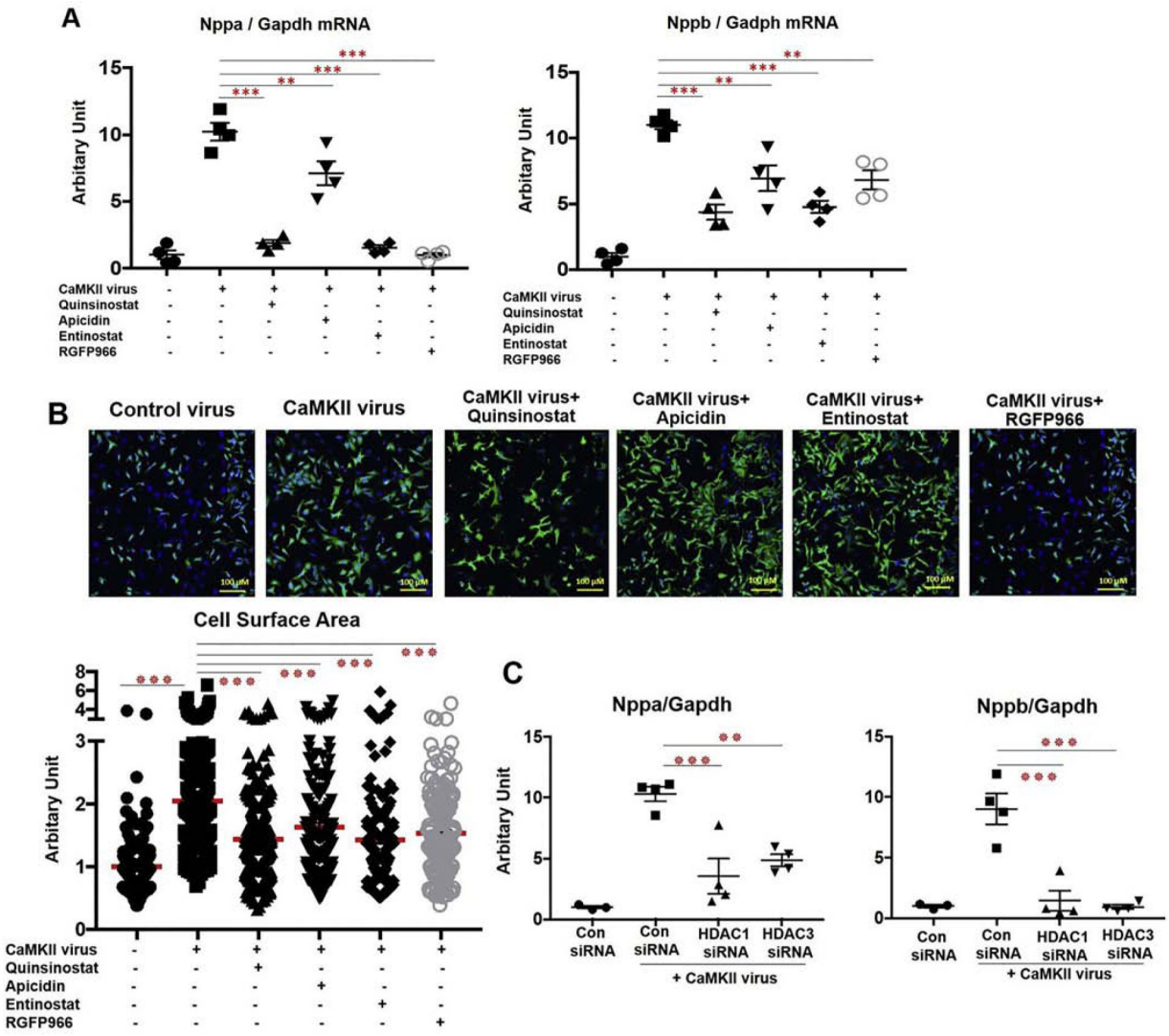

Fig 4. Class I HDACs inhibition attenuates CaMKII overexpression induced cardiac hypertrophy

A. Overexpression of CaMKII induced NRCM hypertrophy which was assessed by ANP/BNP expression. HDAC1 inhibitor Quisinostat, selective class I HDAC inhibitor Apicidin and Entinostat, and HDAC3 specific inhibitor RGFP966 prevented CaMKII elicited hypertrophy (n=4, ** *P<0.01*, *** *P<0.001*). **B.** Consistently, Quisinostat, Apicidin, Entinostat and RGFP966 attenuated CaMKII-induced hypertrophy measured by cell surface area (n=150–285). **C.** In addition to class I HDAC inhibitors, siRNA against HDAC1 and HDAC3 mitigated CaMKII-induced cardiomyocyte hypertrophy.

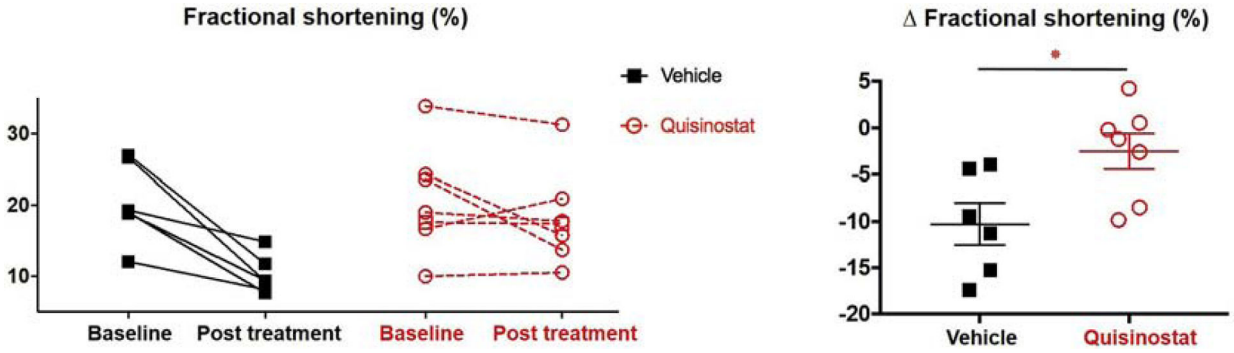

Fig 5. Quisinostat administration slowed down the progression of heart failure in CaMKIIδc-tg mice

Vehicle (n=6) vs Quisinostat (n=7), Baseline fractional shortening (FS): 20.5±2.3% vs 20.7±2.8%, after treatment for two weeks: 10.2±1.1% vs 18.2±2.5%, ΔFS −10± 2.248% vs −2.523±1.901. *$P<0.05$

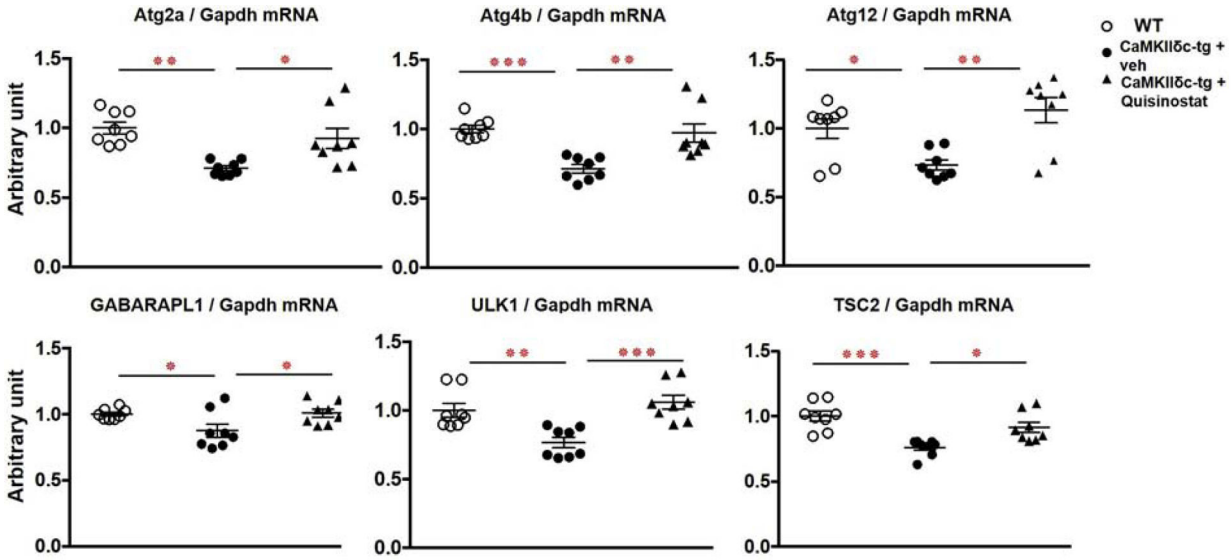

Fig 6. HDAC1 inhibitor improves downregulated autophagy genes in CaMKII-tg mice hearts

Autophagy genes, Atg2a, Atg4b, Atg12, GABARAPL1, ULK1 and TSC2 were downregulated in CaMKII-tg mice hearts compared to WT controls. HDAC1 inhibitor Quisinostat administration recovered the suppressed expression of these autophagy related genes toward WT levels in CaMKII-tg mice. (n=8, **P*<0.05, ***P*<0.01, ****P*<0.001)

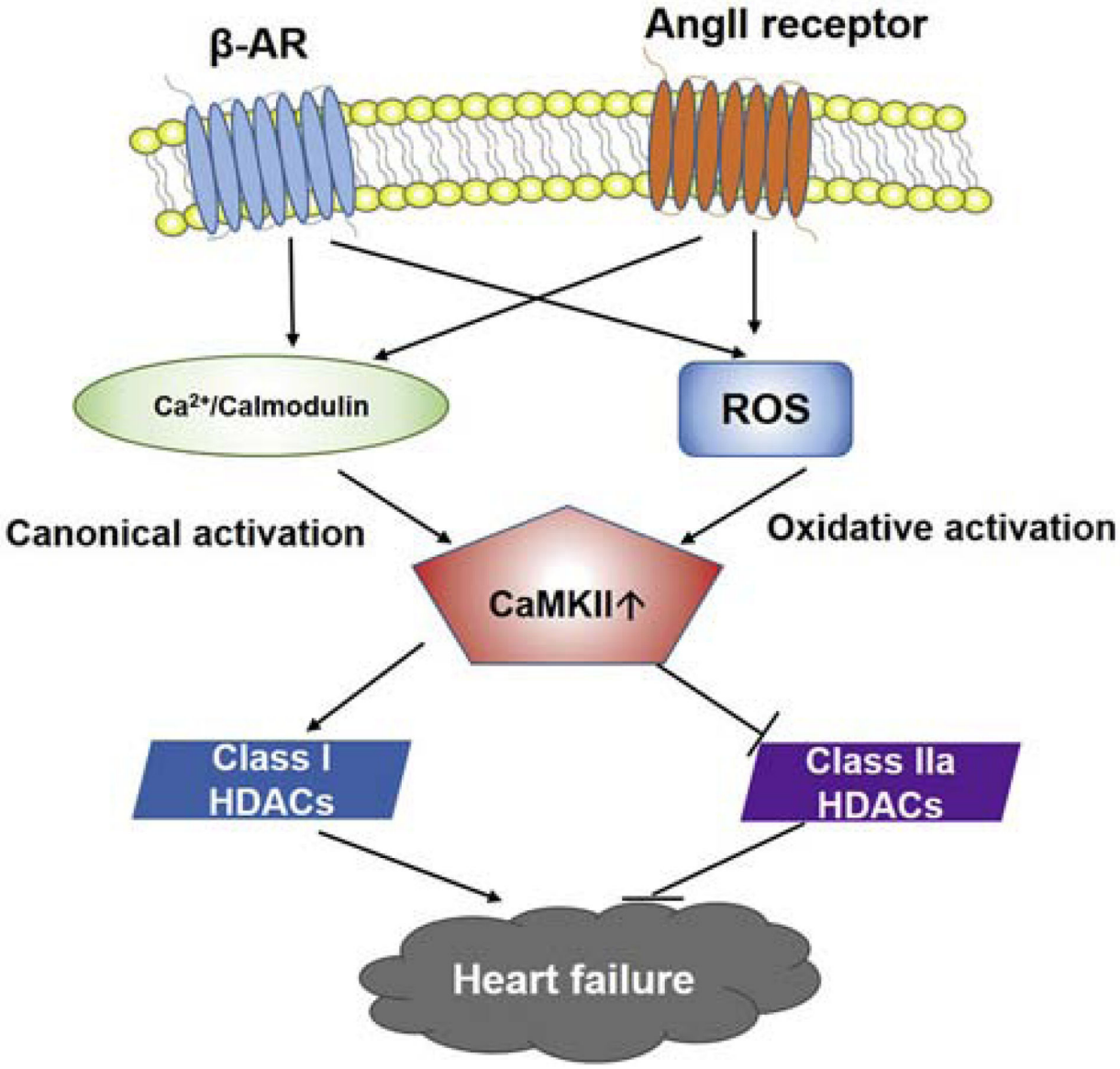

Fig 7. CaMKII promotes heart failure by orchestrating two opposing classes of HDACs

Both CaMKII and Class I HDACs play an important role in many cardiac disease other than heart failure, including ischemia reperfusion injury[40–45], diabetic cardiomyopathy[22, 46, 47] and atrial fibrillation[48–50]. Our findings provide further mechanistic insight of these diseases process and also will facilitate the translation of HDAC inhibitors into clinical use for these diseases. Importantly, both CaMKII and Class I HDACs are widely expressed in many cell types beyond the cardiac myocyte and are implicated in many diseases other than cardiac diseases. Our findings will likely apply to these diseases and provide therapeutic opportunities.