Review

Derepression of pathological cardiac genes by members of the CaM kinase superfamily

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Abstract

In response to pathologic stresses such as hypertension or myocardial infarction, the heart undergoes a remodeling process that is characterized by myocyte hypertrophy, myocyte death and fibrosis, resulting in impaired cardiac function and heart failure. Cardiac remodeling is associated with derepression of genes that contribute to disease progression. This review focuses on evidence linking members of the Ca\(^{2+}/\)calmodulin-dependent protein kinase (CaMK) superfamily, specifically CaMKII, protein kinase D (PKD) and microtubule associated kinase (MARK), to stress-induced derepression of pathological cardiac gene expression through their effects on class IIa histone deacetylases (HDACs). © 2006 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

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1. Introduction

Pathological cardiac remodeling is accompanied by diverse alterations in myocyte gene expression. For example, stress signaling in the adult heart results in up-regulation of dormant “fetal cardiac genes” encoding embryonic isoforms of proteins that govern growth, contractility, calcium handling and energetics, with concomitant down-regulation of the adult isoforms [1]. This gene reprogramming is contingent on phosphorylation-dependent nuclear export of class IIa histone deacetylases (HDACs), which function as endogenous repressors of cardiac gene expression.

Intense investigation has focused on identification of class IIa HDAC kinases. Over 500 genes in the human genome encode kinases, and this “kinome” is comprised of 7 superfamilies [2]. Molecular, biochemical and pharmacologic investigations have led to the identification of three families of class IIa HDAC kinases: Ca\(^{2+}/\)calmodulin (CaM)-dependent protein kinase II (CaMKII), protein kinase D (PKD) and microtubule affinity-regulating kinase (MARK), all of which possess related catalytic domains and fall within the CaMK superfamily. This review describes the details of investigations defining the unique roles for these enzymes as nuclear HDAC kinases.

2. Histone modifications

A central mechanism for the control of gene expression involves the packaging of DNA into chromatin. The basic unit of chromatin, the nucleosome, comprises DNA wrapped around a histone octamer. Nucleosomes interact to create a dense structure that limits access of transcriptional machinery to gene regulatory elements, thereby repressing gene expression. Chromatin structure can be altered by post-translational modification of \(\varepsilon\)-amino groups of multiple conserved lysine residues within nucleosomal histone tails. Nucleosomal lysines can be acetylated, methylated, ubiquitinated or conjugated to small ubiquitin-like modifier (SUMO), and the composite of these modifications creates a “histone code” that governs the expression level of a given gene [3]. The enzymes that catalyze these modifications are referred to as co-factors (co-activators and co-repressors) since they are recruited to genes by DNA-binding transcription factors.
Histone acetyltransferase (HAT)-mediated transfer of acetyl groups from acetyl coenzyme A to nucleosomal lysines results in charge neutralization of the amino acid, which weakens the interaction of positively charged histone tails with the negatively charged phosphate backbone of DNA, and culminates in chromatin relaxation. Histone acetylation is typically associated with enhanced gene expression through creation of a chromatin environment that is capable of accommodating high molecular weight transcriptional machinery. Several HATs play important roles in regulation of cardiac gene expression, including p300 [4] and Tip60 [5], which interact with key cardiac transcription factors such as GATA4, myocyte enhancer factor-2 (MEF2) and serum response factor (SRF). Pharmacological inhibition of HATs blocks agonist-dependent hypertrophy of cultured cardiomyocytes [6].

The actions of HATs are counterpoised by HDACs, which generally inhibit gene transcription. HDAC-catalyzed removal of acetyl-groups from lysines affects gene expression not only by altering the electrostatic properties of histones, but also by creating an opportunity for the once-acetylated lysine residue to be conjugated to ubiquitin, SUMO or a methyl group. These post-translational modifications alter gene expression, both positively and negatively, by altering protein–protein interactions, often through creation of docking sites, or a “code”, for co-activator/co-repressor complexes [7]. Many non-histone proteins are also regulated by acetylation/deacetylation [8], including GATA4 [9] and MEF2 [10].

3. Linking class IIa HDACs to cardiogenic transcription

3.1. Three HDAC classes

There are multiple mammalian HDACs that fall into three classes on the basis of similarity to yeast transcriptional repressors [11,12]. Class I HDACs (1, 2, 3, 8 and 11) are related to yeast RPD3, class II HDACs (4, 5, 6, 7, 9 and 10) to yeast HDA1, and class III HDACs (SirT1–7) to yeast Sir2. Class II HDACs are further divided into two subclasses, IIa (HDACs 4, 5, 7 and 9) and IIb (HDACs 6 and 10).

Class I HDACs are ubiquitously expressed and control expression of a vast array of genes. In the heart, experiments with small molecule inhibitors have suggested roles for class I HDACs as positive regulators of cardiac hypertrophy [13–16]. Class IIb HDACs appear to govern microtubule dynamics by functioning as tubulin deacetylases [17]. Class III HDACs, also known as sirtuins, are unique in that they require nicotinamide adenine dinucleotide (NAD) for catalytic activity, and thus function as sensors of cellular metabolic state [18]. Class III HDAC activity has been linked to inhibition of cardiac hypertrophy and enhanced cardiomyocyte survival [19,20].

This review focuses on class IIa HDACs, which possess highly conserved amino-terminal extensions of ~500 amino acids (Fig. 1) These extensions impart unique functions to class IIa HDACs by serving as docking sites for sequence-specific DNA-binding factors, transcriptional co-factors, and signaling molecules that couple cues from outside the cell to the genome.

3.2. Class IIa HDACs and MEF2

The notion that class IIa HDACs may regulate cardiac gene expression was originally suggested by the discovery that these proteins interact with members of the MEF2 transcription factor family [21–25]. The transcriptional activity of MEF2 factors is up-regulated in response to pathological stress in the heart [26–29], and ectopic overexpression of constitutively active forms of MEF2 in mouse heart causes dilated cardiomyopathy [30]. Class IIa HDACs are recruited to MEF2 target genes through a highly conserved, 18 amino acid MEF2-binding domain in their amino-terminal extensions (Fig. 1). Ectopic overexpression class IIa HDACs 4, 5 or 9 in cultured cardiac myocytes coordinately suppresses MEF2-dependent transcription and agonist-dependent cardiac hypertrophy [29,31–33]. In contrast, disruption of the gene encoding HDAC9 in mice leads to super-activation of cardiac MEF2 activity [29], and mouse knockouts for HDAC5 [34] or HDAC9 [29] develop exaggerated cardiac hypertrophy in response to pressure overload and spontaneous, pathologic hypertrophy with advancing age. Mice lacking functional genes for HDAC4 [35] or HDAC7 [36] die due to defects in bone and vascular development, respectively, and thus a definitive role for these HDACs in the regulation of cardiac hypertrophy has...
not been established. Nonetheless, the gain- and loss-of-function studies with HDACs 5 and 9 support a role for the class Ila HDAC-MEF2 axis in the control of pathological cardiac gene expression.

3.3. Non-MEF2 targets of class Ila HDACs

Recent data suggest that the ability of class Ila HDACs to block cardiac gene expression is governed not only by direct association with MEF2, but also through indirect interactions with unrelated, stress-activated transcription factors. Class Ila HDACs repress genes regulated by serum response factor (SRF) [37,38], nuclear factor of activated T cells (NFAT) [39] and NK family transcription factors [40], all of which have been implicated in the control of pathological cardiac gene expression [41]. In each case, class Ila HDAC binding to the transcription factor is governed by bridging co-factors. HDAC4 is coupled to NFAT by mammalian relative of DnaJ (Mrj) [39], HDAC5 interacts with SRF via myocardin [38], and CAMTA links HDAC5 to NKX2.5 [40] (Fig. 2).

It remains unclear whether repression of gene expression by class Ila HDACs is dependent on intrinsic deacetylase activity. MITR, a splice variant of HDAC9 that lacks a deacetylase domain, effectively blocks MEF2-dependent transcription [42,43] and cardiac hypertrophy [29]. MITR appears to block gene expression through association with co-repressors, such as C-terminal-binding protein (CtBP) [44] and heterochromatin protein-1 (HP1) [45], as well as interactions with class I HDACs [44]. Studies by Verdin and colleagues have suggested that full-length forms of various kinase signaling networks revealed an unexpected role for PKD in the control of cardiac HDAC5 phosphorylation [33]. Protein kinase C (PKC) was shown to stimulate phosphorylation-dependent nuclear export of HDAC5 in cardiac myocytes, but was incapable of directly

functions of class Ila HDACs. In rodents, myocardial levels of class Ila HDACs do not appear to be reduced in disease states [29,34]. Instead, derepression of class Ila HDAC target genes is accomplished, in part, through nucleo-cytoplasmic shuttling of these transcriptional repressors. The amino-terminal extensions of class Ila HDACs harbor two conserved serine residues that are hypo-phosphorylated in unstimulated cardiac myocytes. In response to stress stimuli, such as agonists that activate G protein coupled receptors (GPCRs), these sites become phosphorylated and bound by the 14-3-3 chaperone protein. Binding of 14-3-3 to class Ila HDACs concomitantly unmasks and masks nuclear export and nuclear localization sequences, respectively, with a net outcome of retention of class Ila HDACs to the cytoplasm, and derepression of downstream target genes.

Signal-dependent nuclear export of class Ila HDACs 4 and 5 has been shown to occur in cultured rat cardiac myocytes treated with endothelin-1, angiotensin II, prostaglandin-F2α, phenylephrine and lysophosphatidic acid (LPA), all of which activate GPCRs that couple to G αq. In contrast, cytokines such as leukemia inhibitory factor and TNFα do not trigger nuclear export of HDAC5, and β-adrenergic receptors (β-ARs), which couple to G αs, selectively affect HDAC4 (see below). Blockade of class Ila HDAC nuclear export through substitution of the phospho-acceptor sites with non-phosphorylatable alanine residues [29,31–33], or treatment of cells with the CRM1 antagonist leptomycin B [48], results in suppression of cardiac hypertrophy. Thus, there has been interest in identifying signaling molecules that control class Ila HDACs, with the notion that it may be possible to manipulate cardiac disease via these factors.

4. PKD is a class Ila HDAC kinase

4.1. Identification of PKD as an HDAC kinase

Initial efforts to identify class Ila HDAC kinases relied on the use of a panel of expression vectors encoding various constitutively active kinases [49,50]. These studies revealed remarkable ability of CaMKI and CaMKIV to stimulate nuclear export of HDAC5 in transfected fibroblasts, and were consistent with findings demonstrating that these kinases disrupt MEF2:HDAC5 interactions and drive MEF2-dependent transcription in adult mouse heart [22,28]. However, results of experiments attempting to link endogenous CaMKI/IV signaling to HDAC5 regulation in cardiomyocytes were equivocal, and suggested the existence of distinct class Ila HDAC kinases.

Studies employing cultured cardiac myocytes, adenovirus encoding GFP-tagged HDAC5 and inhibitors/agonists of various kinase signaling networks revealed an unexpected role for PKD in the control of cardiac HDAC5 phosphorylation [33]. Protein kinase C (PKC) was shown to stimulate phosphorylation-dependent nuclear export of HDAC5 in cardiac myocytes, but was incapable of directly

Fig. 2. Repression of cardiac genes by class Ila HDACs. Pathological cardiac gene expression is activated by sequence-specific DNA-binding transcription factors, including MEF2, Nkx2-5, SRF and NFAT. Class Ila HDACs directly bind MEF2 and suppress expression of downstream target genes. Class Ila HDACs indirectly associate with Nkx2-5, SRF and NFAT via bridging co-factors, which are CAMTA, myocardin and Mrj, respectively.
phosphorylating the HDAC. Instead, PKC stimulated HDAC5 phosphorylation through activation of PKD, a known downstream effector kinase.

4.2. Three PKD family members

Three PKD isoforms (1, 2 and 3) make up a family of highly related serine/threonine kinases [51]. PKD1 was discovered in 1994 and was also referred to as PKCθ due to the presence of amino-terminal diacylglycerol (DAG)-binding domains resembling those of PKC [52,53]. However, the catalytic domain of PKD1 is divergent from those of PKC isoforms, and more closely related to that of CaMK. Hence, PKD1, 2 and 3 are now classified as members of the CaMK superfamily [2]. The similarity between the catalytic domains of PKD and CaMK may explain the original observations with ectopically overexpressed CaMKI/IV serving as potent class IIa HDAC kinases.

The three PKD isoforms are highly homologous, consisting of ~900 amino acids, with two amino-terminal cysteine-rich domains (CRDs) that mediate binding to DAG, an internal pleckstrin homology domain (PH) and carboxy-terminal catalytic domains (Fig. 3A). The CRD and PH domains appear to serve auto-inhibitory functions [54,55]. Each PKD isoform is capable of phosphorylating HDAC5 [56] as well as the other class IIa HDACs (4, 7 and 9) [33,57–59] on the serines that are targeted by 14-3-3 (Fig. 1), suggesting the potential for redundant control of class IIa HDACs by PKD family members. Indeed, siRNA knockdown of PKD1 expression in cultured cardiomyocytes blunts but does not eliminate HDAC5 nuclear export [60]. In addition, gene targeting in chicken B lymphocytes demonstrated the need for disruption of both PKD1 and PKD3 in order to block HDAC5 phosphorylation in response to antigen receptor signaling [61].

4.3. Signal-dependent control of PKD activity

There is exact correlation between hypertrophic agonists that trigger nuclear export of HDAC5 in cardiac myocytes and those that activate PKD signaling. PKD is activated by the above-mentioned agonists for GPCRs that couple to Gαq, but not by stimulation of Gαq-coupled β-ARs [60,62]. Studies in non-myocytes have shown that PKD can be activated by other Gαq-coupled receptors, including those for thrombin, bombesin, gastrin, vasopressin, and by growth factor and antigen receptors [51]. Receptor coupling to heterotrimeric G proteins containing Gαq12/13 subunits has also been shown to activate PKD [63,64]. Studies with the Rho GTPase poison, Clostridium botulinum C3 toxin, have indicated a partial role for Rho family members in the control of GPCR-induced activation of PKD [63]. Thus, it is possible that the cardioprotective effects of statins [65], which block Rho activity via HMG-CoA reductase inhibition, are governed in part by suppression of the PKD-class IIa HDAC axis.

PKD proteins are localized to the cytosol of unstimulated cells but translocate to various subcellular compartments, including plasma membrane, mitochondria and Golgi, in response GPCR stimulation. Furthermore, consistent with a role for PKD in the control of nuclear localized class IIa HDACs, each PKD isoform is imported into the nucleus in response to agonist treatment [66–68]. PKD1 has been shown to undergo CRM1-dependent nuclear export [67].

The canonical pathway for signal-dependent activation of PKD involves PKC-mediated phosphorylation of two serine residues within an activation loop, which is embedded in the PKD catalytic domain. Phosphorylation of these sites, which is easily monitored with the use of a phospho-specific antibody [69], relieves PKD from repression by the amino-terminal PH domain (Fig. 3A) [70]. Ca2+-independent, non-conventional PKCs (nPKCs), PKC delta (δ), epsilon (ε), eta (η) and theta (θ), appear to be the dominant regulators of this process. However, in vascular endothelial cells, Ca2+-
dependent PKCδ plays a role in PKD activation in response to vascular endothelial growth factor (VEGF) treatment [71]. It is interesting to note that PKCδ and ε have been linked to cardiomyocyte hypertrophy and death [72]. Whether PKD is involved as a downstream effector that mediates these effects of PKC signaling is unknown.

Several mechanisms for PKC-independent regulation of PKD have been described. For example, in response to antigen receptor signaling, DAG produced via PLC activates PKD indirectly through stimulation of PKC, and directly, via binding to the amino-terminal CRDs in PKD [73]. A similar mechanism appears to be involved in cardiomyocytes treated with ET-1, where PLC is absolutely required for PKD activation, but PKC is dispensable [60]. The auto-inhibitory PH domain can be targeted by tyrosine kinases. For example, in response to oxidative stress, the Abl kinase phosphorylates a tyrosine residue in the PH domain of PKD, resulting in derepression of PKD activity and induction of the NFκB transcription factor [74,75]. Activation of PKD through direct binding of the βγ subunits of heterotrimeric G proteins to the PH domain has also been described [76].

Although β-AR signaling fails to activate PKD, it should be noted that PKA, a major downstream effector of β-ARs, has been reported to potentiate PKD signaling. Carnegie et al. revealed the existence in adult rat heart of a multi-protein complex containing PKD1, PKCζ, PKA and the PKA anchoring protein, AKAP-Lbx [77]. PKA-mediated phosphorylation of AKAP was shown to result in release of PKC-activated PKD1 from the complex. Thus, β-ARs may alter PKD signaling by controlling the subcellular distribution of the kinase rather than through direct effects on its catalytic activity.

4.4. PKD function in the heart

In rodents, cardiac PKD is activated in response to chronic hypertension, pressure overload mediated by aortic constriction, and infusion of agonists such as norepinephrine [60]. Knockdown of endogenous PKD1 expression with siRNA blunts agonist-dependent hypertrophy of cultured neonatal rat cardiac myocytes [60], whereas ectopic overexpression of constitutively active PKD1 in these cells has been reported to induce hypertrophy [78]. In vivo, cardiomyocyte-specific expression of constitutively active PKD1 causes a brief phase of cardiac hypertrophy, followed by chamber dilation and impaired systolic function [60]. Together, these results suggest a role for PKD in cardiac pathogenesis. It will be important to determine the levels and activation status of PKD isoforms in diseased human heart.

In addition to class IIA HDACs, PKD has been implicated in the phosphorylation of the neuronal integral membrane protein kidins 220 [79], the ras effector RIN1 [80], Golgi-associated phosphatidylinositol 4-kinase III beta [81], type II alpha phosphatidylinositol phosphate (PIP) kinase [82], capsaicin receptor VR1 [83], heat shock protein 27 [84], and cardiac troponin I (cTnI) [85]. Furthermore, PKD can mediate signaling events non-catalytically by recruiting the Rap1 GTPase to the cytoplasmic tail of β1 integrin [86]. The extent to which cardiac phenotypes associated with PKD activation are mediated by class IIA HDAC phosphorylation versus phosphorylation of one or more of these other substrates remains unknown. With regard to cardiac contractility, phosphorylation of cTnl, which inhibits actin–myosin interactions, is of particular interest. PKD phosphorylates the same sites on TnI that are phosphorylated by PKA, resulting in reduced myofilament Ca2+ sensitivity. It is not known whether PKD is also capable of phosphorylating other PKA targets that play a role in excitation–contraction coupling, such as phospholamban, the ryanodine receptor or the L-type calcium channel.

Circumstantial evidence suggests a role for PKD in the control of cardiac fibrosis. In response to stress, cardiac fibroblasts proliferate and contribute to fibrotic myocardial remodeling through secretion of collagen and other components of the extracellular matrix [87]. PKD1 is expressed at high levels in adult cardiac fibroblasts [62], and has clearly been shown to govern proliferation of other cell types [51]. The pro-fibrotic mineralocorticoid, aldosterone, can activate PKD [88], and PKD signaling was recently shown to stimulate aldosterone production in adrenal cells through up-regulation of aldosterone synthase expression [89]. These results suggest the possibility that PKD contributes to a positive feedback loop that promotes cardiac fibrosis.

In addition to their emerging roles in the heart, studies in other tissues have revealed functions for PKD family members in diverse biological processes, including protein trafficking from the trans-Golgi network to the plasma membrane [90], stimulation of cell proliferation [91], protection from apoptosis [92,93] and stimulation of apoptosis [58,59,94]. The varied functions of individual PKD family members have recently been reviewed in detail [51,95].

5. CaMKII is an HDAC4 kinase

As mentioned above, although CaMKI and IV are potent class IIA HDAC kinases, evidence for a link between endogenous CaMKI/IV signaling and regulation of class IIA HDACs in cardiac myocytes is lacking. Another paradoxical finding is that overexpressed, constitutively active CaMKII is unable to drive HDAC5 from the nucleus [31].

Six distinct genes encode members of the CaMK family: CaMKI, CaMKIIα, β, γ, δ, and CaMKIV, among which CaMKIIγ and δ are expressed at highest levels in heart [96]. Unlike CaMKI and IV, which are monomeric, CaMKII forms homo- and heteromeric complexes consisting of 6–12 subunits that assemble into a wheel-like structure [97]. Basal CaMKII activity is low due to the action of a pseudosubstrate-like auto-inhibitory domain, which binds to the active site of the enzyme and precludes its association with ATP and protein substrates. Ca2+/CaM complexes bind to and dislodge the auto-inhibitory domain, allowing for subsequent auto-phosphorylation of this domain, which locks
the enzyme in an active, Ca\(^{2+}\)-independent state [98] (Fig. 3B). Several lines of evidence suggest key roles for CaMKII in the control of pathological cardiac remodeling through effects of Ca\(^{2+}\) handling and gene expression [99]. In addition, overexpression of CaM in mouse heart induces cardiac hypertrophy in association with activation of endogenous CaMKII signaling [100–102]. Details of the regulation and function of CaMKII in the heart are covered extensively elsewhere in this review series.

One explanation for the inability of CaMKII to stimulate HDAC5 nuclear export came from the elegant studies of Backs et al. [31]. HDAC4 was shown to undergo agonist-dependent nuclear export in cultured cardiac myocytes in a manner that was sensitive to small molecule inhibitors of CaMK, while HDAC5 nuclear export was unaffected by these same compounds. Detailed biochemical analyses defined a unique 23 amino-acid CaMKII docking domain present in HDAC4 but absent in HDAC5 and the other class IIa HDACs, HDAC7 and HDAC9. Association of CaMKII with HDAC4 can occur in the nucleus, resulting in enhanced CRM1-mediated nuclear export of HDAC4, or in the cytoplasm, resulting in blockage of HDAC4 nuclear import. Each CaMKII isoform (α, β, δ, γ) is capable of interacting with HDAC4 in vitro. However, the nuclear HDAC4 kinase is likely CaMKIIδb, a splice variant of the β isoform that is able to translocate to the nucleus by virtue of a unique NLS. Interestingly, CaMKII is able to phosphorylate a third, cryptic 14-3-3 docking site in HDAC4 (Fig. 1).

By virtue of this unique docking site, HDAC4 is neutralized by Ca\(^{2+}\) signals that result in activation of CaMKII, such as those that arise following β-AR agonism. However, it should be emphasized that HDAC4 is also efficiently phosphorylated by PKD [57]. These results suggest that HDAC4 and HDAC5 may regulate distinct sets of genes, and that differential derepression of class IIa HDAC target genes is dependent on the nature of stimulus.

In contrast to these findings, some studies with small molecule inhibitors have suggested that CaMKII controls HDAC5 nuclear export, for example, in adult cardiac myocytes [103] and neurons [104,105]. In adult rabbit cardiac myocytes, IP\(_3\) receptors in the nuclear envelope can transduce Ca\(^{2+}\) to a nuclear pool of CaMKII that cooperates with PKD to trigger export of HDAC5 to the cytoplasm. The ambiguous findings with CaMKII and HDAC5 may reflect the ability of HDAC4 to form a stable complex with HDAC5 [31]. Through this mechanism, HDAC4 may indirectly convey CaMKII-derived signals to HDAC5. Alternatively, the results may be due to species differences (rat versus rabbit myocytes) or the use of neonatal versus adult cardiac myocytes.

6. MARK is a class IIa HDAC kinase

6.1. Identification of MARKs as class IIa HDAC kinases

In order to further elucidate the signaling pathways regulating class IIa HDACs, Chang et al. performed a cDNA library expression screen to identify factors that enhance phosphorylation-dependent association of HDAC5 with 14-3-3 [57]. This screen yielded cell surface receptors, including endothelin receptor type A (ET-RA) and the receptor for LPA, and downstream effectors such as RhGTPases and PKD. In addition to PKD, microtubule affinity-regulating kinase-2 (MARK2), also referred to as EMK1 and hPar1, was unexpectedly shown to be a potent class IIa HDAC kinase, both in vitro and in cells.

The four MARK family members (MARK1, 2, 3 and 4) fall within the CaMK superfamily [106–108]. MARKs were originally identified by virtue of their ability to phosphorylate the KXGS motif within the microtubule-binding domain of the neuronal tau protein [109]. MARK2 is able to phosphorylate the same sites on HDAC5 that are targeted by PKD (Fig. 1). These phospho-acceptor sequences on HDAC5 (LRKTAS\(^{259}\)EP and LSRTQS\(^{268}\)SP) are conserved in other class IIa HDACs and are similar to the region of tau that is phosphorylated by MARK. A subsequent study demonstrated that MARK3 is also capable of phosphorylating HDAC7 [110]. MARK3 may function as a basally active class IIa HDAC kinase that preferentially phosphorylates the amino-terminal 14-3-3 target site [110].

6.2. Function and regulation of MARKs

MARKs play key roles in the regulation of microtubule dynamics through phosphorylation of tau, as well as related microtubule-associated proteins (MAPs) 2 and 4. Phosphorylation of these proteins is associated with de-stabilization of the microtubule network, and has been implicated as mechanism underlying neurodegenerative disorders such as Alzheimer’s disease [107,109]. Effects of MARKs on the microtubule cytoskeleton also maintain normal cell polarity [111,112]. MARKs are capable of phosphorylating signal transduction machinery, including the dual-specificity phosphatase Cdc25c [113] and the MAP kinase scaffold KSR1 [114]. Interestingly, analogous to class IIa HDACs, phosphorylation of these substrates by MARK creates docking sites for 14-3-3, in which the +2 position relative to the phospho-site is a proline residue [107].

The function(s) of MARKs in heart is completely unknown. Based on mRNA expression analyses, MARK1 and MARK2 appear to be most abundant in heart [107,109]. Global deletion of the gene encoding MARK2 in mice results in dwarfism, infertility, pleiotropic immune system dysfunction and autoimmunity [115,116]. It will be interesting to determine effects of cardiac selective knockout and/or transgenic overexpression of MARK isoforms on class IIa HDAC function and cardiac remodeling.

Several kinases have been shown to phosphorylate activation loop sites within the amino-terminal catalytic domains of MARK family members, resulting in stimulation of MARK function (Fig. 3C). These so-called MARK Kinases (MARKKs) include the LKB1 tumor suppressor [117–119], which plays a role in governing cellular energy
status by activating AMP-activated protein kinase (AMPK) [120]. In addition, thousand and one-amino acid kinase-1 (TAO-1) [121] and glycogen synthase kinase-3β (GSK-3β) [122] can function as MARKKs to stimulate MARK activity. In contrast, the atypical PKC, PKCλ, which is activated independently of DAG and Ca\(^{2+}\), phosphorylates a site in the spacer region of MARK, resulting in inhibition of MARK catalytic activity [123,124]. With regard to the heart, GSK-3β possesses potent anti-hypertrophic activity [125], and thus it is counterintuitive to posit a role for this kinase as a negative regulator of class IIa HDACs. Indeed, studies with activated GSK-3β and inhibitors of GSK-3β have failed to reveal a role for this kinase in the control of class IIa HDAC nuclear export in cardiomyocytes [33,126].

7. Summary and perspectives

Since the discovery of class IIa HDACs in 1999 and 2000, significant progress has been made toward understanding their biological functions and the mechanisms governing their activity. An integrated model for signal-dependent control of pathological cardiac gene expression by class IIa HDACs is presented (Fig. 4). Advances in this area of research have been met with a plethora of new questions. For example, are all of the kinases described in this review physiologically relevant class IIa HDAC kinases? Definitive classification of “HDAC kinases” requires loss-of-function studies, which are complicated by functional redundancy, both intra-family (e.g., PKD1, 2 and 3) and inter-family (e.g., PKD to MARK to CaMKII). Thus, single gene knockouts or knockdowns may fail to reveal effects, as exemplified by the chicken B lymphocyte experiments, in which compound disruption of PKD1 and PKD3 was needed to alter HDAC5 phosphorylation [61]. Clarification of this issue may be provided by novel small molecules that selectively inhibit distinct class IIa HDAC kinase families.

Why should so many class IIa HDAC kinases exist? The need for multiple class IIa HDAC kinases may reflect diverse biological roles for these transcriptional repressors. For example, PKD may predominate as a class IIa HDAC kinase when cells receive G\(^{s}\)-derived signals for cell growth, if a coordinated effect on class IIa HDAC target genes and sets of other G\(^{s}\)/PKD effector genes are needed to elicit a growth response. In contrast, cardiac remodeling driven by high intracellular Ca\(^{2+}\), which is often accompanied by altered expression of Ca\(^{2+}\) handling machinery and cell death, may arise from selective derepression of HDAC4 target genes and activation of other Ca\(^{2+}\)-regulated signaling modules that are not under the control of PKD.

Are the pathways described in this review “druggable” for cardiac disease? The hypothesis predicts that suppression of class IIa HDAC nuclear export by inhibition of HDAC kinases will result in repression of genes required for pathological cardiac remodeling. A test of this hypothesis will require the use of novel small molecule inhibitors of PKD, CaMK and/or MARK in animal models. Again, determining biological effects of pharmacological manipulation of class IIa HDAC kinases could be complicated by the existence of functionally redundant pathways that lead to class IIa HDAC neutralization. Given the pleiotropic functions of these kinases and of class IIa HDACs, it will be important to determine side effects of such compounds. Clues to potential effects of global HDAC kinase inhibition may be provided by studies with forthcoming mice in which genes for distinct HDAC kinases have been disrupted. Finally, it will be essential to establish whether class IIa HDAC modifications occur in human heart disease, and if so, whether these events are influenced by disease etiology. The combined use of novel chemical–genetic techniques and traditional molecular, biochemical and genetic approaches promises to resolve many of these outstanding issues, and will undoubtedly raise a host new questions.

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