Roles and post-translational regulation of cardiac class IIa histone deacetylase isoforms

Kate L. Weeks and Metin Avkiran

Corresponding author: Professor Metin Avkiran

Address:

Cardiovascular Division

King's College London British Heart Foundation Centre

The Rayne Institute

St Thomas' Hospital

Westminster Bridge Road

London SE1 7EH

United Kingdom

Telephone: 020 7188 3899

Fax number: 020 7188 0970

Email: metin.avkiran@kcl.ac.uk

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Abstract

Cardiomyocyte hypertrophy is an integral component of pathological cardiac remodelling in response to mechanical and chemical stresses in settings such as chronic hypertension or myocardial infarction. For hypertrophy to ensue, the pertinent mechanical and chemical signals need to be transmitted from membrane sensors (such as receptors for neurohormonal mediators) to the cardiomyocyte nucleus, leading to altered transcription of the genes that regulate cell growth. In recent years, nuclear histone deacetylases (HDACs) have attracted considerable attention as signal-responsive, distal regulators of the transcriptional reprogramming that in turn precipitates cardiomyocyte hypertrophy, with particular focus on the role of members of the class IIa family, such as HDAC4 and HDAC5. These histone deacetylase isoforms appear to repress cardiomyocyte hypertrophy through mechanisms that involve protein interactions in the cardiomyocyte nucleus, particularly with pro-hypertrophic transcription factors, rather than via histone deacetylation. In contrast, evidence indicates that class I HDACs promote cardiomyocyte hypertrophy through mechanisms that are dependent on their enzymatic activity and thus sensitive to pharmacological HDAC inhibitors. Although considerable progress has been made in understanding the roles of post-translational modifications (PTMs) such as phosphorylation, oxidation and proteolytic cleavage in regulating class IIa HDAC localization and function, more work is required to explore the contributions of other PTMs, such as ubiquitination and sumoylation, as well as potential cross-regulatory interactions between distinct PTMs and between class IIa and class I HDAC isoforms.

Introduction

Histone deacetylases (HDACs) are an ancient family of enzymes that catalyse the removal of acetyl groups from the ε-amino group of specific acetyl lysine residues within their protein substrates. Deacetylation of histones in nucleosomes induces chromatin condensation, which represses transcription by preventing binding of transcription factors and other components of the transcriptional machinery to gene promoter and enhancer regions. Conversely,

acetylation of histones by histone acetyltransferases (HATs) induces chromatin relaxation, resulting in increased gene transcription. Thus, HDACs and HATs serve as important and opposing epigenetic regulators of gene expression.

Of the four classes of non-sirtuin HDACs (I, IIa, IIb and IV; see Figure 1), class I and IIa are the best studied with regards to cardiac biology and pathology. Genetically modified mouse models and the use of pharmacological HDAC inhibitors in experimental models of cardiovascular disease have revealed important roles for both class I and IIa HDACs in the regulation of cardiac structure and function (see Tables 1 and 2). Administration of small molecule HDAC inhibitors, such as trichostatin A (TSA), SAHA and valproic acid, blocks pathological cardiac changes in a range of experimental settings (see Table 1). For example, administration of TSA two weeks after the induction of pressure overload reversed cardiac hypertrophy in mice (Kee *et al.*, 2006), and administration of SAHA at reperfusion reduced infarct size and improved cardiac function in a rabbit model of ischemia/reperfusion injury (Xie *et al.*, 2014). The pharmacophore of most HDAC inhibitors developed to date contains a zinc-binding group that chelates the zinc ion required for catalytic activity (Bertrand, 2010; McKinsey, 2011). As the principal mechanism by which class IIa HDACs regulate cardiomyocyte hypertrophy is not dependent on their catalytic activity (Zhang *et al.*, 2002a), and class IIa HDACs are relatively insensitive to HDAC inhibitors (Lahm *et al.*, 2007; Bradner *et al.*, 2010), the efficacy of HDAC inhibitors in attenuating pathological cardiac remodelling in animal models is likely a consequence of their inhibition of class I HDAC isoforms. Although we are not aware of conclusive clinical evidence regarding the therapeutic potential of HDAC inhibitors in the context of heart failure, in patients with epilepsy treatment with valproic acid appears to be associated with a reduced risk of myocardial infarction (Olesen *et al.*, 2011).

Class IIa HDACs are endogenous inhibitors of cardiomyocyte hypertrophy and are therefore referred to as “anti-hypertrophic”. Class IIa HDACs suppress cardiomyocyte hypertrophy when localised in the nucleus by repressing the activity of pro-hypertrophic transcription factors, such as members of the myocyte enhancer factor-2 (MEF2) family (Lu *et al.*, 2000; Zhang *et al.*, 2002a),

and by recruiting epigenetic regulators such as class I HDACs and histone methyltransferases to DNA promoter regions (Fischle *et al.*, 2002; Zhang *et al.*, 2002b; Hohl *et al.*, 2013). Nuclear export of class IIa HDACs permits the induction of hypertrophic genes by alleviating their repressive interactions with transcription factors and allowing the recruitment of HATs and histone demethylases. The relative importance of and interplay between class I and class IIa HDAC isoforms in regulating cardiomyocyte hypertrophy and other components of pathological cardiac remodelling such as fibrosis are the subject of intense research. The recent discovery that cardiac class IIa HDACs may regulate the acetylation status and activity of class I HDACs (Eom *et al.*, 2014) reveals a new layer of complexity that warrants further investigation as part of this effort.

HDACs are subject to various post-translational modifications, including phosphorylation, proteolytic cleavage, oxidation, ubiquitination and sumoylation. This review will focus on the roles and regulation of class IIa HDACs in the context of cardiomyocyte hypertrophy and cardiac remodelling, with emphasis on post-translational modifications that alter the function of individual members of this class. For broader reviews covering other HDAC classes and their potential as targets for cardiac pharmacotherapy, the reader is referred to recent review articles from the laboratories of two leading investigators in the field, Timothy McKinsey (McKinsey, 2012) and Joseph Hill (Xie & Hill, 2013).

Class IIa HDACs

The HDAC superfamily can be divided into four classes, based on sequence similarity and functional domains (see Figure 1). Class I and II HDACs are conserved amongst prokaryotes and eukaryotes, while class IV HDACs are present in all organisms except fungi (Gregoretti *et al.*, 2004). Class III HDACs are more commonly known as sirtuins and constitute an unrelated class of NAD-dependent deacetylases (Landry *et al.*, 2000).

The class II HDACs can be further divided into two subclasses. Class IIa includes HDAC4, HDAC5, HDAC7, HDAC9 and a truncated splice variant of HDAC9 known as MEF2-interacting transcriptional repressor (MITR) or HDAC-related protein

(HDRP). Class IIb includes HDAC6 and HDAC10. Unlike class I HDACs, which are widely expressed (Yang *et al.*, 1997; Hu *et al.*, 2000), class IIa HDAC expression is restricted to a subset of tissues and cell types. In humans and mice, expression of HDAC4, HDAC5 and HDAC9 is highest in heart, brain and skeletal muscle (Grozinger *et al.*, 1999; Zhou *et al.*, 2001), whereas HDAC7 expression is restricted to endothelial cells within the heart, lung and skeletal muscle (Kao *et al.*, 2000; Chang *et al.*, 2006). Expression of MITR/HDRP is highest in heart and brain (Zhou *et al.*, 2000; Zhang *et al.*, 2001). Serial analysis of gene expression (SAGE) has identified HDAC5 as the most abundant HDAC transcript in the adult human heart (de Ruijter *et al.*, 2003).

Structure and function of class IIa HDACs

With the exception of MITR/HDRP, which lacks a deacetylase domain, class IIa HDACs consist of a C-terminal deacetylase domain and an extensive N-terminal regulatory domain. Class IIa HDACs have very low deacetylase activity towards native acetyl lysine residues, despite being capable of deacetylating the synthetic substrate trifluoro acetyl lysine *in vitro* (Bradner *et al.*, 2010). This is due to the replacement of a conserved tyrosine residue with a histidine residue within the deacetylase domain of class IIa HDACs, which markedly impairs catalytic activity (Lahm *et al.*, 2007). It has been proposed that the deacetylase domain of class IIa HDACs functions as a binding domain, as class IIa HDACs bind acetyl lysine substrates with comparable affinity to class I and IIb HDACs, despite being unable to deacetylate these substrates (Bradner *et al.*, 2010). It has also been suggested that deacetylase activity associated with class IIa HDACs purified from cells or tissue most likely arises from the presence of HDAC3 in class IIa HDAC repressor complexes (Fischle *et al.*, 2002).

As illustrated in Figure 2, the regulatory domain of class IIa HDACs contains a MEF2 binding motif, a nuclear localisation signal (NLS), and several conserved serine residues that act as docking sites for 14-3-3 proteins when phosphorylated. The best characterised HDAC kinases in the context of cardiac biology and pathology are Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein kinase D (PKD). However, in various cell types and *in vitro* settings, class IIa HDACs appear to be substrates also for protein kinase A (PKA),

G protein-coupled receptor kinase-5, microtubule affinity-regulating kinases, salt-inducible kinases and AMP-dependent protein kinases (Chang *et al.*, 2005; Dequiedt *et al.*, 2006; Berdeaux *et al.*, 2007; McGee *et al.*, 2008; Ha *et al.*, 2010; Zhang *et al.*, 2011; Walkinshaw *et al.*, 2013a). Association with 14-3-3 proteins masks the NLS and sequesters HDACs in the cytoplasm, following unveiling of a C-terminal nuclear export sequence and transport out of the nucleus by the nuclear export receptor CRM1 (Grozinger & Schreiber, 2000; McKinsey *et al.*, 2001; Harrison *et al.*, 2004). Nucleo-cytoplasmic shuttling appears to be the primary mechanism regulating class IIa HDAC function, as exclusion from the nucleus prevents the repressive interaction of HDACs with transcription factors, resulting in increased transcription of target genes (Miska *et al.*, 1999; Lu *et al.*, 2000; Harrison *et al.*, 2004). In the case of MEF2, nuclear export of class IIa HDACs permits interaction with the HAT, E1 binding protein p300 (p300), which promotes pro-hypertrophic gene transcription via its effects on histone acetylation and the formation of enhanceosomes (Wei *et al.*, 2008; He *et al.*, 2011). p300 is also capable of directly acetylating MEF2, which enhances DNA binding and transcriptional activity (Ma *et al.*, 2005).

Regulation of cardiac remodelling by class IIa HDACs

The majority of the evidence implicating class IIa HDACs in the regulation of cardiomyocyte hypertrophy and cardiac remodelling comes from genetically modified mouse models (see Table 2), most of which have been generated in the laboratory of Eric Olson, and *in vitro* studies in primary and immortalised cell lines. Heterologously expressed HDAC4 and HDAC5 are predominantly nuclear, but accumulate in the cytoplasm upon exposure to pro-hypertrophic stimuli, such as the α_1-adrenergic receptor agonist phenylephrine (PE) and endothelin-1 (ET-1) (Harrison *et al.*, 2004; Vega *et al.*, 2004a; Ago *et al.*, 2008; Peng *et al.*, 2009; Backs *et al.*, 2011; Haworth *et al.*, 2012; Chang *et al.*, 2013). Blocking class IIa HDAC nuclear export with CRM1 inhibitors, such as leptomycin B or its derivatives, or by mutation of the HDAC 14-3-3 docking sites to non-phosphorylatable alanine residues, prevents cardiomyocyte hypertrophy (Harrison *et al.*, 2004; Vega *et al.*, 2004a; Monovich *et al.*, 2009), providing strong

evidence that such nuclear export is required for the induction of the hypertrophic response.

Mice globally deficient in either HDAC5 or HDAC9 were viable, but developed cardiac hypertrophy even in the absence of an imposed stress by approximately 8 months of age (Zhang *et al.*, 2002a; Chang *et al.*, 2004). In contrast, compound deletion of both *Hdac5* and *Hdac9* resulted in embryonic lethality due to haemorrhage and ventricular defects (Chang *et al.*, 2004). The small percentage of double-knockout mice that survived to adulthood were severely growth retarded and displayed significant cardiac hypertrophy, demonstrating that HDAC5 and HDAC9 have important functions in embryonic and postnatal cardiac development.

Mice with individual deletion of *Hdac5* or *Hdac9* displayed an exaggerated hypertrophic response to pressure overload, induced by constriction of the thoracic aorta, suggesting that these HDAC isoforms function to limit cardiac enlargement following haemodynamic overload (Zhang *et al.*, 2002a; Chang *et al.*, 2004). Both genotypes also displayed profound hypertrophic growth compared with wild-type littermates when crossed with transgenic mice expressing activated calcineurin, an important transducer of pro-hypertrophic signalling (Molkentin *et al.*, 1998). The findings that HDAC5 and HDAC9 knockout mice respond similarly to hypertrophic stimuli, and that HDAC5/9 double-knockout mice are embryonically lethal, suggest that these isoforms are activated by similar signalling pathways and have overlapping functions. Functional redundancy of class IIa HDACs has been observed also in the context of fibre type switching in skeletal muscle; deletion of individual class IIa HDAC isoforms had no effect on the proportion of type I and type II fibres in mouse soleus muscle, whereas heterozygous or homozygous deletion of multiple isoforms (*Hdac4/5*, *Hdac5/9* or *Hdac4/5/9*) increased the percentage of type I fibres (Potthoff *et al.*, 2007).

Mice with global deletion of *Hdac4* die prior to weaning due to severe growth retardation resulting from the premature ossification of developing bones (Vega *et al.*, 2004b). Although no obvious cardiac abnormalities were observed in these mice, it was impossible to examine the effects of *Hdac4* deletion on stress-

induced cardiac hypertrophy as the mice died prior to adulthood. Mice with cardiomyocyte-specific deletion of *Hdac4* have since been generated (Hohl *et al.*, 2013), but the effects of hypertrophic stress on their cardiac phenotype have not been described in detail to date. In their recent collaborative study, the laboratories of Johannes Backs and Christoph Maack have used these mice, as well as tissue samples from failing and non-failing human myocardium, to investigate the epigenetic regulation of *Nppa* and *Nppb*, foetal genes encoding the atrial and B-type natriuretic peptides ANP and BNP, which are re-expressed during cardiac hypertrophy and heart failure (Hohl *et al.*, 2013). Their findings support the hypothesis that class IIa HDACs repress MEF2-dependent gene transcription by recruiting the methyltransferase SUV39H1 and the adaptor protein heterochromatin protein 1 (HP1) to promoter regions, as part of a corepressor complex (Zhang *et al.*, 2002b). Nuclear export of HDAC4 in response to elevated cardiac load may increase expression of ANP and BNP, not via effects on histone acetylation but via histone demethylation by JMJC domain-containing demethylases, following dissociation of HP1 and SUV39H1 (Hohl *et al.*, 2013). Whether similar mechanisms operate in HDAC4-mediated regulation of other hypertrophic genes, or in the regulation of ANP and BNP expression by other class IIa HDACs, has not been reported.

Mice with global deletion of *Hdac7* die during embryogenesis due to cardiovascular defects (Chang *et al.*, 2006). Conditional deletion of *Hdac7* in endothelial cells phenocopied global deletion, whereas mice with conditional deletion of *Hdac7* in cardiomyocytes were viable (Chang *et al.*, 2006). Thus, any effect of HDAC7 on cardiac remodelling is likely to arise predominantly from its function in endothelial cells. In this context, PKD-mediated phosphorylation of HDAC7 following VEGF stimulation of endothelial cells led to nuclear export of HDAC7, increased MEF2 activity and enhanced angiogenesis in an *ex vivo* assay (Ha *et al.*, 2008a). Nuclear export of HDAC5 was shown to have a similar pro-angiogenic effect (Ha *et al.*, 2008b). As insufficient angiogenesis is a critical determinant of the transition from compensatory cardiac hypertrophy to decompensated heart failure (Shiojima *et al.*, 2005), it is likely that class IIa HDACs regulate pathological cardiac remodelling via their functions in multiple cell types, including endothelial cells, and not just in cardiomyocytes. It follows

from this that any new therapies targeted at class IIa HDAC nucleocytoplasmic shuttling may need to exhibit cell specificity, in order to limit cardiomyocyte hypertrophy but not compromise angiogenesis during cardiac remodelling.

Post-translational modifications regulating class IIa HDAC function

Phosphorylation

HDAC5 contains at least 17 phospho-acceptor residues that are conserved across species, which suggests that phosphorylation is a fundamental post-translational modification regulating HDAC5 folding and function (Greco *et al.*, 2011). The best characterised HDAC5 phosphorylation sites are Ser259 and Ser498, which flank the NLS, and Ser279, which lies within the NLS. Homologous sites are present in HDAC4 (see Figure 2) and HDAC9. As mentioned previously, phosphorylation of Ser259 and Ser498 in HDAC5 by CaMKII or PKD induces nuclear export (McKinsey *et al.*, 2000a; McKinsey *et al.*, 2000b; Vega *et al.*, 2004a; Backs *et al.*, 2006; Backs *et al.*, 2008). In contrast, phosphorylation of Ser279 by protein kinase A (PKA) has been proposed to promote nuclear retention (Ha *et al.*, 2010; Chang *et al.*, 2013; Walkinshaw *et al.*, 2013b), possibly by inducing a conformational change that prevents binding of 14-3-3 proteins at phosphorylated Ser259 and Ser498 (Ha *et al.*, 2010).

Expression and activity of CaMKIIδ and PKD are elevated in failing human myocardium (Bossuyt *et al.*, 2008), and both kinases contribute to pathological remodelling in rodents (Fielitz *et al.*, 2008; Backs *et al.*, 2009). HDAC4 contains a unique CaMKII docking site that promotes interaction with activated CaMKII and subsequent phosphorylation of Ser467 and Ser632 (Backs *et al.*, 2006). HDAC5 lacks a CaMKII docking site, but can be phosphorylated by CaMKII when bound to HDAC4 via its coiled-coil domain (Backs *et al.*, 2008). Nuclear CaMKIIδ phosphorylates HDAC5 following $InsP_3$-induced Ca^{2+} release from the nuclear envelope (Wu *et al.*, 2006), while cytosolic CaMKIIδ could maintain HDACs in a phosphorylated state once exported to the cytoplasm (Backs *et al.*, 2006). PKD1, which is predominantly cytosolic under basal conditions, associates with the sarcolemma upon PE stimulation, then rapidly translocates to the nucleus where it phosphorylates HDAC5 (Bossuyt *et al.*, 2011).

Intriguingly, recent evidence suggests that nuclear export of HDAC5 may occur independently of increases in Ser259 and Ser498 phosphorylation. Stimulation with the β-adrenergic receptor (β-AR) agonist isoprenaline induced nuclear export of HDAC5 in adult rat ventricular myocytes, but this was accompanied by reduced phosphorylation of the relevant 14-3-3 docking sites (Haworth *et al.*, 2012). The mechanism(s) responsible for reduced HDAC5 phosphorylation in this experimental setting is not yet known. Of interest, all three subunits of the heterotrimeric protein phosphatase 2A (PP2A) holoenzyme co-immunoprecipitate with HDAC5 when stably expressed in HEK293 cells (Greco *et al.*, 2011) and PP2A has been found to dephosphorylate HDAC4 *in vitro* (Paroni *et al.*, 2008). Whether PP2A-mediated dephosphorylation contributes to reduced HDAC5 phosphorylation at Ser259 and Ser498 in response to β-adrenergic stimulation in cardiac myocytes, and the role of any such dephosphorylation in regulating the nuclear localisation of HDAC5, require further investigation.

The phosphorylation status of Ser279 appears to be a key determinant of HDAC5 localisation, as mutation to a phosphomimetic aspartic acid residue promoted nuclear accumulation and rendered HDAC5 resistant to nuclear export induced by PE or ET1 stimulation in neonatal rat and adult rabbit cardiac myocytes (Ha *et al.*, 2010; Chang *et al.*, 2013). Furthermore, mutation of Ser279 to alanine promoted cytoplasmic accumulation in U2OS cells, consistent with the hypothesis that phosphorylation of Ser279 promotes nuclear retention (Greco *et al.*, 2011). However, no change in basal distribution was observed with a Ser279Ala HDAC5 mutant in adult rabbit cardiac myocytes (Chang *et al.*, 2013). In the same study, it was proposed that phosphorylation of Ser279 by β-AR-mediated activation of PKA promotes nuclear import/retention of HDAC5, as isoprenaline and forskolin, an activator of adenylyl cyclase, induced nuclear import of wild-type HDAC5, but had no effect on the localisation of the Ser279Ala mutant (Chang *et al.*, 2013). As noted above, the β-AR agonist isoprenaline has been reported to induce HDAC5 nuclear export and increased MEF2 activity in adult rat ventricular myocytes (Haworth *et al.*, 2012). Thus the regulation of HDAC5 localisation and function by β-AR stimulation and the roles of

phosphorylation/dephosphorylation at regulatory serine residues appear to also warrant additional investigation.

With regards to adrenergic regulation of class IIa HDACs, very recent evidence suggests that increased HDAC4/5 phosphorylation and consequent MEF2 activation following endothelin-1 stimulation may occur primarily through stimulation of α_1- and β_1-ARs on cardiomyocytes, following activation of presynaptic ET_A receptors and subsequent inhibition of noradrenaline reuptake into sympathetic nerve terminals (Lehmann *et al.*, 2014). This observation suggests that there is significant crosstalk between different neurohormonal stimuli in the regulation of class IIa HDACs, not only downstream of pertinent GPCRs within cardiomyocytes but also through neuronal presynaptic receptors.

Proteolytic cleavage

An additional mechanism by which PKA may regulate the function of HDAC4 has been described in a study by the laboratories of Backs and Olson (Backs *et al.*, 2011). HDAC4 contains a cleavage site (Tyr201), which is not present in other class IIa HDACs (see Figure 2), and activation of PKA leads to HDAC4 cleavage at this site by an as yet unidentified serine protease, resulting in the production of an N-terminal fragment (HDAC4-NT) that accumulates in the nucleus where it represses MEF2 activity. HDAC4-NT was detected in hearts from wild-type mice following isoprenaline administration over a four-hour period, but not in hearts from mice lacking the catalytic α subunit of PKA, confirming that PKA-dependent generation of HDAC4-NT can occur *in vivo* downstream of β-AR stimulation. It has been suggested that this mechanism may allow cardiomyocytes to exhibit differential hypertrophic responses to acute adrenergic activation in physiological stress situations and to sustained neurohormonal stimulation during prolonged periods of cardiac stress in disease (Backs *et al.*, 2011). Accordingly, during acute β-AR stimulation, PKA-mediated generation of HDAC4-NT would reign in MEF2 activity, attenuating hypertrophic gene transcription. In settings of sustained neurohormonal stimulation, involving also other mediators such as ET-1, angiotensin II and reactive oxygen species (see Oxidation section below), CaMKII- and PKD-mediated, phosphorylation-dependent as well as phosphorylation-independent mechanisms of class IIa HDAC nuclear export

would predominate, leading to MEF2 activation and pathological cardiac remodelling. In this context, selective stimulation of β-ARs is sufficient to induce cardiomyocyte hypertrophy and cardiac remodelling (Osadchii, 2007) and β-AR antagonists are clinically proven therapies for chronic heart failure (Bristow, 2011). It seems likely, therefore, that any HDAC4-NT-mediated anti-hypertrophic consequences of β-AR stimulation are indeed short lasting, and/or that mechanisms that are independent of class IIa HDACs make a predominant contribution to cardiomyocyte hypertrophy and cardiac remodelling during chronic β-AR stimulation.

There is evidence that HDAC4 can be cleaved also by caspase-2 and caspase-3 at Asp289 (Paroni *et al.*, 2004). The resulting N-terminal fragment augments apoptosis via the repression of serum response factor (SRF) and Runx2 (Liu *et al.*, 2004; Paroni *et al.*, 2004; Paroni *et al.*, 2007; Backs *et al.*, 2011). Whether this proteolytic cleavage event occurs in the heart and has a functional role during cardiac stress responses has not been explored.

Oxidation

Junichi Sadoshima and colleagues have identified oxidation as a novel phosphorylation-independent post-translational modification regulating subcellular localisation of HDAC4 in cardiomyocytes (Ago *et al.*, 2008) and suggested that nicotinamide adenine dinucleotide phosphate oxidase 4 (Nox4) plays an important role in generating the relevant oxidative signal (Matsushima *et al.*, 2013). Although the precise mechanism of oxidation-induced nuclear export is unknown, it has been proposed that formation of an intra-molecular disulphide bond between two conserved cysteine residues (Cys667 and Cys669 in HDAC4; see Figure 2) disrupts zinc coordination, leading to a conformational change that exposes the nuclear export signal to CRM1 (Ago *et al.*, 2008). HDAC5 appears to be regulated in a similar manner, as isoprenaline-induced nuclear export of HDAC5 was blocked by overexpression of the disulphide oxidoreductase protein thioredoxin-1 or in the presence of the antioxidant N-acetylcysteine (Haworth *et al.*, 2012).

Ubiquitination

Ubiquitination plays a critical role in cell homeostasis, regulating protein turnover by targeting proteins for degradation by the proteasome. Reversible ubiquitination of protein substrates also serves an important role in signal transduction and has been shown to modulate gene expression in several cardiac disease settings (Portbury *et al.*, 2012). Although HDAC ubiquitination has not been studied in the heart, a study in skeletal muscle suggests that ubiquitination is an important post-translational modification that regulates class IIa HDAC function (Potthoff *et al.*, 2007). In this study, ubiquitination and subsequent degradation of class IIa HDACs promoted the expression of slow twitch fibre-specific genes by alleviating their repressive interaction with MEF2 (Potthoff *et al.*, 2007). Proteasomal inhibition in mice resulted in increased HDAC4 and HDAC5 expression and reduced MEF2 activity in various skeletal muscles, while Cre-mediated deletion of multiple class IIa HDACs resulted in a switch from fast- to slow-twitch fibres, demonstrating that ubiquitination is an important post-translational mechanism that maintains appropriate levels of HDAC expression. Whether ubiquitination of class IIa HDACs is important in cardiac biology is yet to be explored, but this seems likely.

Sumoylation

Sumoylation, the covalent attachment of small ubiquitin-like modifier (SUMO) proteins to target proteins, is emerging as an important post-translational mechanism regulating cardiovascular homeostasis (Kho *et al.*, 2011; Wang *et al.*, 2011). HDAC4 is sumoylated in HeLa cells, possibly as it enters the nucleus through the nuclear pore complex (Kirsh *et al.*, 2002). Interestingly, interaction with HDAC4 or HDAC5 potentiates the sumoylation of MEF2, which renders MEF2 transcriptionally inactive, and ablation of the SUMO conjugation site in HDAC4 dampens the repressive effect of HDAC4 on MEF2-dependent gene transcription (Gregoire & Yang, 2005; Zhao *et al.*, 2005). Whether altered sumoylation of class IIa HDACs plays a role in the pathogenesis of cardiovascular disease has not been investigated.

Summary

Figure 2 summarises the current state of knowledge regarding the opposing roles of class IIa and class I HDAC isoforms in regulating pathological cardiac remodelling and outlines the multiple PTMs that have already been shown to regulate class IIa HDAC function, as well as those that warrant further investigation, in this context. Over the past decade or more, substantial evidence has accumulated that class IIa HDAC isoforms such as HDAC4 and HDAC5 are important regulators of cardiomyocyte hypertrophy and pathological cardiac remodelling, principally through their repressive effects on MEF2 transcription factor activity when enriched in the nucleus. Importantly, the subcellular localisation, integrity and MEF2 interaction of class IIa HDAC isoforms appear to be regulated by multiple post-translational mechanisms such as phosphorylation, oxidation and proteolytic cleavage, and potentially sumoylation, ubiquitination and proteasomal degradation. Greater understanding of such mechanisms, and potential cross-regulatory interactions between them, may allow the development of new therapeutic approaches towards harnessing the "anti-hypertrophic" actions of class IIa HDAC isoforms under conditions of chronic mechanical and neurohormonal stress and thereby attenuating pathological cardiac remodelling. This is particularly relevant given recent reports that class IIa HDACs can additionally regulate the expression and activity of "pro-hypertrophic' class I HDACs (Spallotta *et al.*, 2013; Eom *et al.*, 2014). A greater understanding of the interplay between different HDAC classes at different stages of disease, as well as the interplay between HDACs and other epigenetic regulators, such as bromodomain and extra-terminal acetyl-lysine reader proteins (Anand *et al.*, 2013; Spiltoir *et al.*, 2013), is likely to aid the development of new targeted therapies for the prevention or reversal of pathological cardiac remodelling and heart failure.

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Figure legends

Figure 1: A) The histone deacetylase (HDAC) family is comprised of four classes. Class II HDACs are further categorised into subclasses. Class I and class IIa HDAC isoforms are the best characterised with regards to cardiomyocyte hypertrophy and cardiac remodelling. This review focuses primarily on the Class IIa subclass. B) HDAC4 and HDAC5 are highly homologous, sharing a conserved MEF2 binding domain (BD), a nuclear localisation signal (NLS), a deacetylase domain and a nuclear export sequence (NES). Phosphorylation by protein kinases at conserved serine residues within the N-terminal region is a key determinant of subcellular localisation. Oxidation of conserved cysteine residues within the deacetylase domain is a phosphorylation-independent post-translational mechanism that can also affect HDAC4/5 localisation. Proteolytic cleavage of HDAC4 at a unique site results in the generation of an N-terminal fragment that represses MEF2 activity. HDAC4 also contains a CaMKII binding domain, which is not present in HDAC5.

Figure 2: A schematic of the current state of play and avenues for future investigation. Class I and class IIa HDACs are now recognized as important and opposing regulators of pathological cardiac remodelling, through mechanisms that are either dependent on (class I) or independent of (class IIa) histone acetyl lysine deacetylase activity. The figure also illustrates various post-translational modifications (PTMs) of class IIa HDACs whose role in regulating pathological cardiac remodelling is either relatively well established (black) or requires further investigation (blue). Potential interactions and cross-regulation between different PTMs remain unknown, and recent evidence (Eom *et al.*, 2014) for deacetylation of HDAC2 (a class I HDAC) by HDAC5 (a class IIa HDAC) should trigger further investigation of regulatory crosstalk between distinct HDAC family members.

Table 1: HDAC inhibitors attenuate pathological remodelling in experimental models of cardiac injury

Inhibitor	Isoform selectivity	Effect of inhibitor on cardiac phenotype
Trichostatin A (TSA)	Pan HDACi	Blunted cardiac hypertrophy induced by chronic isoprenaline infusion in mice (Kook *et al.*, 2003) Blunted cardiac hypertrophy induced by ascending aortic banding in mice and rats (Kee *et al.*, 2006) Prevented cardiac hypertrophy induced by chronic angiotensin II infusion in mice (Kee *et al.*, 2006) Reversed established cardiac hypertrophy induced by ascending aortic banding in mice (Kee *et al.*, 2006) Blunted left ventricular hypertrophy and attenuated cardiac fibrosis induced by transverse aortic banding in mice (Kong *et al.*, 2006) Reduced infarct size in mouse models of ischemia/reperfusion injury (Granger *et al.*, 2008; Xie *et al.*, 2014) Reduced infarct size and improved functional parameters in Langendorff isolated perfused mouse hearts (Zhang *et al.*, 2010) Attenuated pathological remodelling and improved survival in a mouse model of myocardial infarction (Zhang *et al.*, 2012)
Valproic acid (VPA)	Weak class I HDACi	Blunted cardiac hypertrophy induced by ascending aortic banding in mice (Kee *et al.*, 2006) Prevented cardiac hypertrophy induced by chronic angiotensin II infusion in mice (Kee *et al.*, 2006) Attenuated left ventricular remodelling and improved systolic function in a rat model of myocardial infarction (Lee *et al.*, 2007) Reduced blood pressure and prevented the development of left ventricular hypertrophy and fibrosis in a genetic rat model of hypertension (Cardinale *et al.*, 2010) Attenuated right ventricular hypertrophy and fibrosis induced by pulmonary artery banding in rats (Cho *et al.*, 2010) Attenuated left and right ventricular hypertrophy in a rat model of pulmonary hypertension induced by monocrotaline injection (Cho *et al.*, 2010) Prevented/reversed cardiac hypertrophy and fibrosis in DOCA-salt hypertensive rats (Kee *et al.*, 2013)
Scriptaid	Pan HDACi	Blunted left ventricular hypertrophy induced by transverse aortic banding in mice (Kong *et al.*, 2006) Reduced infarct size in a mouse model of ischemia/reperfusion injury (Granger *et al.*, 2008)
SAHA	Pan HDACi	Reduced blood pressure and attenuated left ventricular hypertrophy and fibrosis in DOCA-salt hypertensive rats (Iyer *et al.*, 2010) Reduced infarct size and improved systolic function in a mouse model of ischemia/reperfusion injury (Xie *et al.*, 2014) Reduced infarct size and improved systolic function in rabbits when administered prior to ischemia/reperfusion injury or at reperfusion (Xie *et al.*, 2014)
Apicidin	Class I HDACi	Blunted left ventricular hypertrophy, attenuated cardiac fibrosis and improved systolic function in mice subjected to transverse aortic banding (Gallo *et al.*, 2008)
SK-7041	Class I/pan HDACi	Prevented cardiac hypertrophy induced by ascending aortic banding in mice (Kee *et al.*, 2006)

Table 2: Loss- and gain-of-function HDAC mouse models

Isoform	Mouse model	Reported cardiac phenotype	References
HDAC1	Global knockout	Embryonic lethality between E9.5 and E10.5 due to proliferation defects.	(Lagger *et al.*, 2002)
	Global knockout	Embryonic lethality by E9.5.	(Montgomery *et al.*, 2007)
	Cardiomyocyte-specific knockout	No gross cardiac abnormalities basally. Comparable hypertrophic response to chronic isoprenaline administration and transverse aortic constriction (TAC) as littermate controls.	(Montgomery *et al.*, 2007)
	Cardiomyocyte-specific transgenic	No evidence of cardiac hypertrophy at 2-3 months of age.	(Trivedi *et al.*, 2007)
HDAC2	Global knockout	Increased cardiomyocyte hyperplasia during perinatal period. Resistant to isoprenaline- and TAC-induced cardiac remodelling. Resistant to cardiac hypertrophy induced by transgenic expression of the homeobox gene, *Hop*.	(Trivedi *et al.*, 2007)
	Global knockout	Neonatal lethality due to ventricular defects. Increased cardiomyocyte hyperplasia and apoptosis in P1 hearts. Bradycardia.	(Montgomery *et al.*, 2007)
	Cardiomyocyte-specific knockout	No gross cardiac abnormalities. Comparable hypertrophic response to chronic isoprenaline administration as littermate controls.	(Montgomery *et al.*, 2007)
	Cardiomyocyte-specific transgenic	Developed pathological cardiac hypertrophy by 8 weeks of age. Further increase in heart mass when crossed with transgenic mice overexpressing the homeobox gene, *Hop*.	(Trivedi *et al.*, 2007)
HDAC1/2	Double cardiomyocyte-specific knockout	Postnatal lethality due to the development of dilated cardiomyopathy and cardiac arrhythmias. Phenotype attributed to dysregulation of genes encoding ion channels and sarcomeric proteins.	(Montgomery *et al.*, 2007)
HDAC3	Global knockout	Embryonic lethality by E9.5.	(Montgomery *et al.*, 2008)
	Cardiomyocyte-specific knockout	Developed significant left ventricular hypertrophy, atrial enlargement, interstitial fibrosis and systolic dysfunction by 12 weeks of age. Phenotype attributed to dysregulation of metabolic genes.	(Montgomery *et al.*, 2008)
	Cardiomyocyte-specific transgenic	Significant thickening of the ventricular walls and diminished lumen volume at birth due to increased cardiomyocyte proliferation. Normalisation of heart morphology by 2-3 months of age. Similar hypertrophic response to chronic isoprenaline treatment as wild-type littermates.	(Trivedi *et al.*, 2007; Trivedi *et al.*, 2008)
HDAC4	Global knockout	Lethality prior to weaning due to severe growth retardation resulting from premature ossification of developing bones. No obvious cardiac phenotype.	(Vega *et al.*, 2004b)
	Cardiomyocyte-specific knockout	Cardiac phenotype has not been extensively described. Normal dP/dt_{max}, dP/dt_{min} and cardiac output in isolated working hearts. Elevated *Nppa* expression (marker of pathological cardiac hypertrophy) compared with wild-type littermates.	(Hohl *et al.*, 2013)
HDAC5	Global knockout	Developed cardiac hypertrophy by 8 months of age. Displayed exaggerated hypertrophic response to TAC and transgenic expression of activated calcineurin. Similar hypertrophic response to wild-type littermates in response to chronic isoprenaline administration.	(Chang *et al.*, 2004)
HDAC6	Global knockout	Protected from developing systolic dysfunction in response to chronic angiotensin II infusion or TAC, despite the same degree of left ventricular hypertrophy and fibrosis as wild-type littermates.	(Demos-Davies *et al.*, 2014)
HDAC7	Global knockout	Embryonic lethality due to cardiovascular defects.	(Chang *et al.*, 2006)
HDAC8	Global knockout	Perinatal lethality due to skull defects. No obvious cardiovascular abnormalities.	(Haberland *et al.*, 2009)
HDAC9	Global knockout	Developed cardiac hypertrophy by 8 months of age. Displayed exaggerated hypertrophic response to TAC and transgenic expression of activated calcineurin. Similar hypertrophic response to wild-type littermates in response to chronic isoprenaline infusion.	(Zhang *et al.*, 2002a; Chang *et al.*, 2004)
HDAC5/9	Double global knockout	High incidence of embryonic and perinatal lethality due to haemorrhages and ventricular defects. Cardiac hypertrophy was observed in the small percentage of double knockout mice that survived until adulthood.	(Chang *et al.*, 2004)

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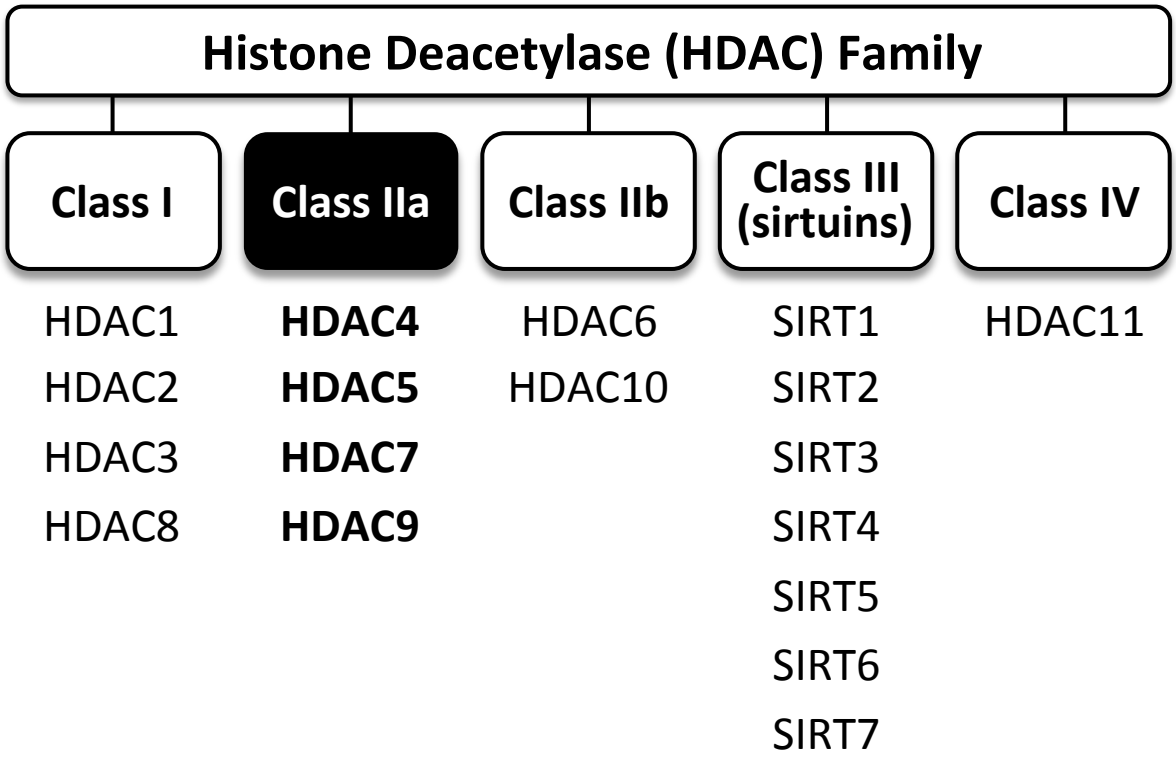
A



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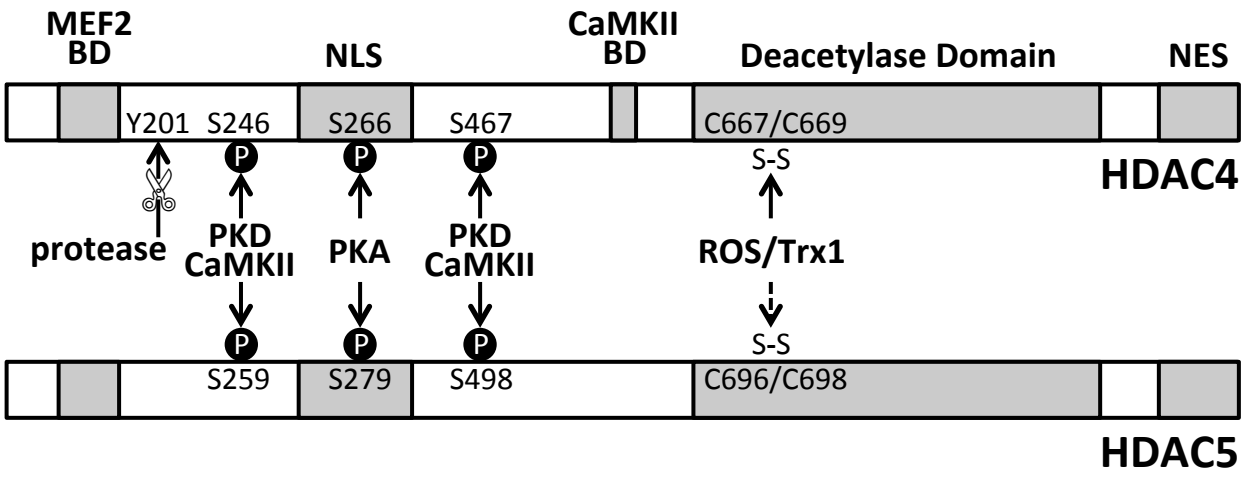
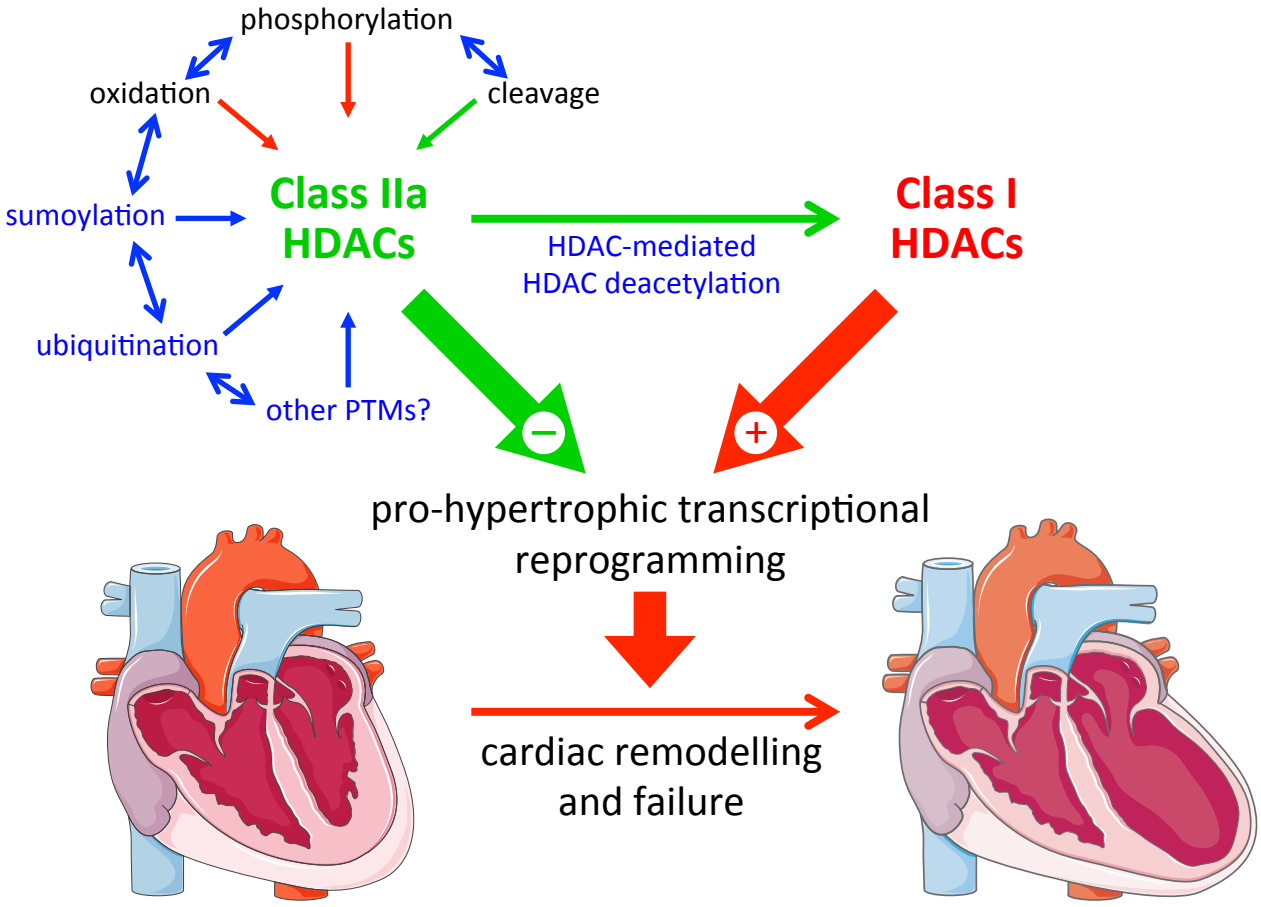


HDAC4	HDAC5
Y201: SSDPR**Y**WYGKT	–
S246: LRKTA**S**EPNLK	**S259:** LRKTA**S**EPNLK
S266: AERRS**S**PLLRR	**S279:** AERRS**S**PLLRR
S467: LGRTQ**S**APLPQ	**S498:** LSRTQ**S**SPLPQ
C667/C669: LKHQ**C**T**C**GSSS	**C696/C698:** LKHQ**C**M**C**GNTH


phosphorylation
oxidation
cleavage
sumoylation
Class IIa HDACs
HDAC-mediated HDAC deacetylation
Class I HDACs
ubiquitination
other PTMs?
pro-hypertrophic transcriptional reprogramming
cardiac remodelling and failure