HDACs and Hypertrophy, Kinases and Cancer

Berdymammet Hojayev, PhD; Joseph A. Hill, MD, PhD

In the course of daily life, the heart reacts to alterations in workload demand with changes in contractility and beating rate; over time, robust changes in ventricular volume and mass take place. In the context of organism development, athleticism, or pregnancy, the cardiac growth response is normal and adaptive. In the context of pathological triggers, however, cardiac remodeling is ultimately maladaptive and a discrete milestone in disease pathogenesis. The end result is heart failure, a syndrome with ≈50% 5-year mortality, rivaling the most lethal cancers.

Plasticity of any tissue entails the complex interplay of protein synthesis, degradation, and posttranslational modification. In general, both anabolic and catabolic pathways are activated, and intricate cascades of protein modifications and protein degradation are triggered. The importance of reversible protein acetylation, a dynamic regulator of function that rivals protein phosphorylation in terms of ubiquity and importance, has come to the fore. Acetylation-dependent regulatory circuits are robust, operating in concert with pathways controlled by other posttranslational modifications to govern homeostasis and stress responsiveness. In the case of histones, acetylation of the ε-NH₂ group of lysine residue side chains leads to relaxation of chromatin structure, enhanced accessibility to DNA-binding proteins, and consequent activation of transcription.

Protein acetylation is regulated by histone acetyltransferases and histone deacetylases (HDACs). These HDACs, in turn, are categorized into 4 classes. Class I HDACs (1, 2, 3, 8) are expressed ubiquitously, and consist mainly of a deacetylase domain that catalyzes hydrolytic release of the acetyl side chains, leading to relaxation of chromatin structure, enhanced accessibility to DNA-binding proteins, and consequent activation of transcription.

Histone deacetylase 2 in Heart Disease

In the current issue of *Circulation*, Eom et al provide evidence for kinase-dependent governance of HDAC2 activity in cardiac myocytes. This work builds on a literature, significant elements of which derive from prior studies from this same group, implicating HDAC2 in cardiac stress responsiveness. First, mice lacking enzymatically active HDAC2 manifest blunted hypertrophic responses to aortic constriction or isoproterenol infusion; in addition, HDAC2 transgenic mice develop hypertrophy. Inactivation of the gene coding for HDAC2 triggers early postnatal lethality. At the same time, other work has uncovered evidence of the redundancy of HDAC1 and HDAC2 actions in cardiac morphogenesis, growth, and contractility. Indeed, HDAC1 and HDAC2 share ≈80% amino acid homology and often coexist in transcriptional complexes.

Histone deacetylases, including HDAC2, are governed by posttranslational events that control their subcellular localization, binding partners, and enzymatic activities. HDAC2 protein, in particular, is regulated by serine phosphorylation, lysine ubiquitylation, tyrosine nitration, cysteine nitrosylation, and binding by the heat shock protein HSP70. In addition, the oncogenic protein casein kinase 2 (CK2) phosphorylates HDAC2, thereby promoting enzymatic activity and interactions with transcriptional repressors, transcription factors, and promoter regions.

Histone deacetylases have been shown to be phosphorylated at a C-terminal domain, and this phosphorylation is associated with enhanced enzymatic activity. To probe for a possible role in the heart, Eom et al tested for HDAC2 phosphorylation in cardiac hypertrophy. Of 4 potential phosphorylation sites, serines 394 and 411 were phosphorylated in the setting of hypertrophic stimulation. Further studies suggested that phosphorylation of serine 394, but not 411, is required for enzymatic activity in the context of growth cues.
HDAC2 overexpression elicits hypertrophic growth both in vivo and in vitro, and Eom et al extend this by showing that alanine substitution at serine 394 abolished cell growth. Then, recognizing that CK2 is capable of phosphorylating HDAC2, the investigators used CK2 inhibitors and RNA interference targeting CK2, finding that each was sufficient to suppress HDAC2 phosphorylation in neonatal cardiomyocytes. Similarly, phosphorylation and enzymatic activity of recombinant GST-HDAC2 were both increased on exposure to lysates from isoproterenol-treated hearts, a response that was blocked by CK2 inhibitors.

The experimental strategy used to this point relied on preventing phosphorylation of HDAC2. Moving forward, it will be important to evaluate the effects of promoting phosphorylation with, for example, mutations that mimic residue phosphorylation. Does an S394E mutant manifest enhanced enzymatic activity and more hypertrophy? Data along these lines will be required to firmly establish a conclusion that CK2-mediated phosphorylation of HDAC2 triggers cardiac growth.

Overexpression of CK2α1 in cardiomyocytes elicited protein synthesis and presumably cell growth, arguing for sufficiency of the pathway. Inhibition of CK2 activity or CK2 protein abundance each suppressed hypertrophic growth of neonatal cardiomyocytes, arguing for necessity. Importantly, cardiomyocyte CK2 activity was increased when cells were exposed to growth triggers. Inhibition of HDAC2 by either small interfering RNA or a small molecular inhibitor blunted CK2α1-induced increases in protein synthesis, consistent with a model where HDAC2 is a critical downstream target of CK2.

Finally, in a series of in vivo studies, the investigators engineered transgenic mice that overexpress CK2α1 driven by the cardiomyocyte-specific α myosin heavy chain promoter. These mice developed hypertrophy and fetal gene reactivation, responses that were augmented when these transgenics were crossed with HDAC2 overexpressors. Conversely, cardiac remodeling was not exacerbated when CK2α1 transgenic mice were crossed with mice overexpressing S394A-mutated HDAC2. In aggregate, these studies provide evidence for a signaling axis where hypertrophic stimuli provoke CK2 translocation into the nucleus, consequent phosphorylation of HDAC2 at serine 394 (and other targets), ultimately leading to cardiomyocyte growth.

Analogy to Cancer

Over the years, similarities between heart failure and cancer have been highlighted, noting the numerous commonalities of signal transduction, inexorability of disease progression, and prognosis. Given that cardiac myocytes are largely incapable of reentering the cell cycle, signaling events that provoke tumor cell proliferation provoke cell growth, metabolic derangements, and cell death in the heart. On the basis of the work reported here, a CK2-HDAC2 signaling axis may be another example of a conserved mechanism linking these 2 devastating disorders.

First, both CK2 and HDAC2 are aberrantly expressed in cancer, and high levels of HDAC2 are independent markers of poor prognosis in patients with a variety of tumors. Second, precedent exists for the therapeutic application of HDAC inhibitors in oncology and in preclinical trials of heart disease. In oncology, HDAC inhibitors upregulate cell-cycle inhibitors (eg, p21), blocking both growth and proliferation of tumors. Indeed, a number of clinical trials are underway to test HDAC inhibitors in patients with cancer, and 2 compounds are FDA-approved; similar trials in patients with heart disease have been proposed.

Third, bringing these 2 together, CK2-dependent phosphorylation of HDAC2 has been implicated in cancer. However, a point of divergence emerges here; whereas CK2 and HDAC2 are each upregulated in cancer, there is no evidence that either alone can trigger tumorigenesis. On the other hand, overexpression of either CK2 or HDAC2 in cardiac myocytes is sufficient to induce hypertrophy.

As noted, prior work has documented that HDAC2 is phosphorylated by CK2, and HDAC2 phosphorylation is required for enzymatic activity. Interestingly, CK2-dependent phosphorylation of HDAC2 at serine 394 has been demonstrated in vascular smooth muscle cells exposed to retinoic acid receptor agonists, leading to deacetylation of Klf5. HDAC2 phosphorylation by CK2 is also induced by hypoxia in tumor cells, although the specific site of phosphorylation has not been mapped. In all these contexts, HDAC2 phosphorylation was associated with stimulus-induced nuclear translocation of CK2.

In aggregate, these studies suggest that activation of HDAC2 by CK2-dependent phosphorylation at serine 394 is a signaling axis common to cardiomyocytes, vascular smooth muscle cells, and cancer cells. If true, targeting this molecular cascade is particularly attractive, in comparison with the wide array of other CK2 and/or HDAC2 effectors.

Moving Forward

Just as with any good study, this work raises important new questions. Among them, what is the molecular target(s) of phosphorylation-dependent HDAC2 activation? How is it that relatively modest increases in the phosphorylation of HDAC2 are associated with a robust hypertrophic growth response? How does simple overexpression of a serine 394 phosphorylation mutant blunt hypertrophy when the wild-type alleles remain expressed?

Nitro-oxidative stress triggers CK2-dependent phosphorylation and tyrosine nitration of HDAC2, leading to its degradation in the proteasome. Does CK2 perform an analogous, dual action on cardiac HDAC2? As CK2 targets a host of downstream effectors, do any of these elements participate in stress-induced cardiac remodeling? The fact that CK2α1 transgensics manifest hypertrophy even when coexpressed with phosphorylation-mutant HDAC2 suggests this may be the case. What is the cognate phosphatase that antagonizes this pathway, and does it suppress hypertrophy?

CK2 phosphorylates ARC (apoptosis repressor with caspase recruitment domain), a reaction that inhibits hypertrophy. During hypertrophy, ARC phosphorylation is diminished because of the reduced activity of oxidatively modified CK2. However, this study and another recent article support a positive role for both CK2α1 and CK2α2 in hypertrophy. Whereas CK2α1 works through HDAC2...
activation, CK2α2 downregulates p27, an antihypertrophic cell-cycle regulator.\textsuperscript{18} CK2α1 knockout mice are embryonic lethal, indicating that this isoform is not compensated by the presence of CK2α2.\textsuperscript{19} Further elucidation of these kinase isoforms will be enlightening.

HDAC2 is phosphorylated only by CK2; unlike HDAC1, it is not targeted by protein kinase A or protein kinase G.\textsuperscript{10} Eom et al demonstrate that CK2 phosphorylates only serines 394 and 411. Thus, it will be important to determine events governing the phosphorylation of serines 422 and 424, which are required for basal HDAC2 activity.

Small-molecule HDAC inhibitors regulate global gene expression and have proven effective and well tolerated in the treatment of cancer; (pre)clinical trials are underway in inflammatory and heart diseases. New, isoform-specific molecules are being developed. Whereas several studies have demonstrated a significant role of HDAC2 in heart hypertrophy, its role in conditions of heart failure, arrhythmias, and ischemia/reperfusion injury remains unknown. Do HDAC inhibitors work through inhibition of HDAC2, and what is the phosphorylation status of serine 394 under these conditions? Are mice expressing S394A-mutant HDAC2 resistant to developing heart failure and arrhythmias?

In summary, pathways active in cancer cause great mischief in the heart. Even as these insights emerge, however, we lag behind our colleagues in oncology in translating them to novel therapies with clinical impact. By providing tantalizing evidence for a CK2-HDAC2 signaling axis in cardiac hypertrophy, studies such as that by Eom et al add to a growing literature implicating HDACs in disease pathogenesis and raise yet further the prospect of targeting them for therapeutic gain.

Acknowledgments
We thank members of the Hill laboratory for valuable suggestions and comments.

Sources of Funding
This work was supported by grants from the NIH (HL-075173; HL-080144; HL-090842), ADA (0640084N), ADA (7-08-MN-21-ADA), AHA-Jon Holden DeHaan Foundation (0970518N), and the Fondation Leducq.

Disclosures
None.

References

Key Word: Editorials  |  hypertrophy
HDACs and Hypertrophy, Kinases and Cancer
Berdymammet Hojayev and Joseph A. Hill

Circulation. 2011;123:2341-2343; originally published online May 16, 2011; doi: 10.1161/CIRCULATIONAHA.111.032441
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/123/21/2341

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/