

## STATE-OF-THE-ART REVIEW

# Epigenetics in Cardiac Hypertrophy and Heart Failure



Chia-Feng Liu, PhD,<sup>a</sup> W.H. Wilson Tang, MD<sup>a,b</sup>

## JACC: BASIC TO TRANSLATIONAL SCIENCE CME/MOC/ECME

This article has been selected as this month's *JACC: Basic to Translational Science* CME/MOC/ECME activity, available online at <http://www.acc.org/jacc-journals-cme> by selecting the *JACC* Journals CME/MOC/ECME tab.

### Accreditation and Designation Statement

The American College of Cardiology Foundation (ACCF) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) and the European Board for Accreditation in Cardiology (EBAC) to provide continuing medical education for physicians.

The ACCF designates this Journal-based CME/MOC/ECME activity for a maximum of 1 AMAPRA Category 1 Credit or 1 EBAC Credit. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Successful completion of this CME activity, which includes participation in the evaluation component, enables the participant to earn up to 1 Medical Knowledge MOC point in the American Board of Internal Medicine's (ABIM) Maintenance of Certification (MOC) program. Participants will earn MOC points equivalent to the amount of CME credits claimed for the activity. It is the CME activity provider's responsibility to submit participant completion information to ACCME for the purpose of granting ABIM MOC credit.

**Epigenetics in Cardiac Hypertrophy and Heart Failure** will be accredited by the European Board for Accreditation in Cardiology (EBAC) for 1 hour of External CME credits. Each participant should claim only those hours of credit that have actually been spent in the educational activity. The Accreditation Council for Continuing Medical Education (ACCME) and the European Board for Accreditation in Cardiology (EBAC) have recognized each other's accreditation systems as substantially equivalent. Apply for credit through the post-course evaluation.

### Method of Participation and Receipt of CME/MOC/ECME Certificate

To obtain credit for *JACC: Basic to Translational Science* CME/MOC/ECME, you must:

1. Be an ACC member or JACBTS subscriber.
2. Carefully read the CME/MOC/ECME-designated article available online and in this issue of the *journal*.

3. Answer the post-test questions. At least 2 questions provided must be answered correctly to obtain credit.
4. Complete a brief evaluation.
5. Claim your CME/MOC/ECME credit and receive your certificate electronically by following the instructions given at the conclusion of the activity.

**CME/MOC/ECME Objective for This Article:** Upon completion of this activity, the learner should be able to: 1) identify the inheritance pattern of hypertrophic cardiomyopathy; 2) discuss the recommendations for screening first degree family members of patients with inherited cardiomyopathies (such as hypertrophic cardiomyopathy); and 3) compare the molecular mechanisms for epigenetic gene regulation.

**CME/MOC/ECME Editor Disclosure:** CME/MOC/ECME Editor L. Kristin Newby, MD, is supported by research grants from Amylin, Bristol-Myers Squibb Company, GlaxoSmithKline, Sanofi, Verily Life Sciences (formerly Google Life Sciences), the MURDOCK Study, NIH, and PCORI; receives consultant fees/honoraria from BioKier, DemeRx, MedScape/The-Heart.org, Metanomics, Philips Healthcare, Roche Diagnostics, CMAC Health Education & Research Institute; serves as an Officer, Director, Trustee, or other fiduciary role for the AstraZeneca HealthCare Foundation and the Society of Chest Pain Centers (now part of ACC); and serves in another role for the American Heart Association and is the Deputy Editor of *JACC: Basic to Translational Science*.

**Author Disclosures:** Dr. Tang is supported by grants from the National Institutes of Health (NIH) and the Office of Dietary Supplements (R01DK106000, R01HL126827), Collins Family Fund, and Wortzman Family Fund. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Dr. Tang has a financial relationship with Sequana Medical Inc. and MyoKardis Inc. Dr. Liu has reported that she does not have any relationships relevant to the contents of this paper to disclose.

**Medium of Participation:** Online (article and quiz).

### CME/MOC/ECME Term of Approval

Issue Date: December 2019

Expiration Date: November 30, 2020

From the <sup>a</sup>Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio; and the <sup>b</sup>Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic, Cleveland, Ohio. Dr. Tang is supported by grants from the National Institutes of Health (NIH) and the Office of Dietary Supplements (R01DK106000, R01HL126827), Collins Family Fund, and Wortzman Family Fund. The content is solely the responsibility of the authors and does

# Epigenetics in Cardiac Hypertrophy and Heart Failure

Chia-Feng Liu, PhD,<sup>a</sup> W.H. Wilson Tang, MD<sup>a,b</sup>

## HIGHLIGHTS

- Epigenetic mechanisms associated with the pathological process of cardiac hypertrophy and failure include DNA methylation, post-modification of histones, ATP-dependent chromatin conformation and remodeling, and non-coding RNAs.
- Systemic- and cardiac-epigenetic mechanisms may both influence the disease processes of cardiac hypertrophy and failure.
- Identifying vital epigenetic machinery in cardiac diseases may facilitate developing personalized therapy for HF.

## SUMMARY

Heart failure (HF) is a complex syndrome affecting millions of people around the world. Over the past decade, the therapeutic potential of targeting epigenetic regulators in HF has been discussed extensively. Recent advances in next-generation sequencing techniques have contributed substantial progress in our understanding of the role of DNA methylation, post-translational modifications of histones, adenosine triphosphate (ATP)-dependent chromatin conformation and remodeling, and non-coding RNAs in HF pathophysiology. In this review, we summarize epigenomic studies on human and animal models in HF. (J Am Coll Cardiol Basic Trans Science 2019;4:976-93) © 2019 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The word “epigenetics” is composed of a Greek prefix, “epi,” meaning “upon,” “on,” or “around” and the word “genetics.” Therefore, epigenetics can be defined as the mechanism that affects heritable changes in gene expression and function without alternating the sequence of the genome. It plays fundamental roles in regulating the architecture of chromatin and gene expression at various molecular levels for maintaining cell identity and controlling cell differentiation, which are critically important for normal development and diseases. Changes of the epigenome are hallmarks of various cancers and several potentially molecules have been identified as potential new drug therapies.

The study of epigenetics in cardiovascular diseases is a relatively new field. Heart failure (HF), being the leading cause of death worldwide, occurs when the myocardium has long been recognized to undergo structural and functional remodeling. Such processes may be the cause and/or consequence of various

genomic and transcriptional reprogramming of the cardiomyocytes and other nearby cells. Hence, better understanding of how epigenetic regulations are involved in HF may open a new perspective for translational research into new diagnostic tools as well as novel strategies in drug design and discovery.

Epigenetic regulation can be classified at 4 different molecular levels: 1) DNA methylation, 2) post-translational modifications of histones, 3) adenosine triphosphate (ATP)-dependent chromatin conformation and remodeling, and 4) non-coding RNAs. In this review, we will summarize the recent advances and provide an overview of the epigenomics studies focusing on HF in humans and animal models.

## DNA METHYLATION: A POTENTIAL BIOMARKER FOR HF

The methylation of DNA in eukaryotes predominantly occurs at the fifth carbon of the pyrimidine ring of the

not necessarily represent the official views of the NIH. Dr. Tang has a financial relationship with Sequana Medical Inc. and MyoKardis Inc. Dr. Liu has reported that she does not have any relationships relevant to the contents of this paper to disclose. The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* [author instructions page](#).

Manuscript received February 26, 2019; revised manuscript received May 29, 2019, accepted May 30, 2019.

## ABBREVIATIONS AND ACRONYMS

<b>BET</b>	= bromodomain
<b>EZH2</b>	= Enhancer of zeste homolog 2
<b>HAT</b>	= histone acetyltransferase
<b>HDAC</b>	= histone deacetylase
<b>HDM</b>	= histone demethylase
<b>HF</b>	= heart failure
<b>HMT</b>	= histone methyltransferase
<b>lnc-RNAs</b>	= long ncRNAs
<b>PRC2</b>	= polycomb repressor complex 2
<b>PTMs</b>	= post-translational modifications
<b>TAD</b>	= topologically associating domains
<b>TMAO</b>	= trimethylamine N-oxide

cytosine (5mC) and followed by a guanine dinucleotide where cytosine and guanine are separated by a phosphate (CpG). DNA methylation plays a crucial role in gene regulation, particularly on transcriptional repression that depends on where methylation is located. Increased methylation of CpG-enriched regions, known as CpG islands (CGI) at the promoter region of genes, is often associated with gene silencing, whereas methylated CpG found in the gene body is usually related to gene activation (1). In many pathological conditions, particularly in the cancer genome, alteration of DNA methylation of genes is the first epigenetic hallmark associated with the disease process (2). For example, a typical signature in many types of cancer cells is the hypermethylation at CGI of tumor suppressor genes causing transcriptional silencing of these genes. The silencing of these tumor suppressor genes affects the progression of the tumorigenesis (3).

The pioneering genome-wide studies on DNA methylation in the failing human myocardium were performed a decade ago (4,5). Movassagh et al. first demonstrated that a large population of CGI and promoters were hypomethylated in the end-stage failing heart, and such differential DNA methylation patterns correlated with differential expression of angiogenic factors (4). Using methylated DNA immunoprecipitation followed by sequencing, they determined that the differential DNA methylation between non-failing and end-stage failing hearts did not occur evenly across the genome, but concentrated at promoter CGIs, intragenic CGIs, and gene bodies (5). In 2013, Haas et al. identified a set of candidate genes with altered DNA methylation status that may be involved in HF by using a lower-resolution method (6). Among these candidate genes, 2 genes displayed differential gene expression in dilated cardiomyopathy (DCM): adenosine receptor A2A (*Adora2A*) and lymphocyte antigen 75 (*Ly75*). Interestingly, when they validated these 2 genes by knockdown either *Ly75* or *Adora2A* using morpholino in zebrafish, both morphants (zebrafish mutants) developed severe HF similar to that observed in humans (6).

A more extensive human study on DNA methylation in HF was published recently. Meder et al. generated a genome-wide DNA methylation profile in patients with DCM and in donor left-ventricular biopsies and whole peripheral blood using a high-resolution epigenomic-wide method with a large cohort (7). They identified 59 CpG loci with significant changes in DNA

methylation in the myocardium of patients with DCM compared with clinical controls. Among these CpG loci, 29 and 30 were hypomethylated and hypermethylated in DCM, respectively. Using multi-omics approaches, they linked a subset of 517 epigenetic loci with DCM and cardiac gene expression. By further examining the methylome of peripheral blood cells, a differential display of 217 methylation sites between controls and patients with DCM was observed. Moreover, when they compared methylome between myocardium and peripheral blood cells, they identified distinct epigenetic methylation patterns that are conserved between 2 tissues. For example, the *NPPA* (natriuretic peptide A, also known as ANP) and *NPPB* (natriuretic peptide B, also known as BNP) loci were demethylated in DNA in heart tissues and peripheral blood cells from patients with DCM (7).

Using a cell-sorting technique coupled with a whole-genome bisulfite sequencing approach, Gilsbach et al. showed that only a few genomic regions within gene body of 6 genes exhibited differential DNA methylation between non-failing and failing human cardiomyocytes (8). However, those genes did not express differentially between the 2 groups. Additionally, the global DNA methylation patterns are not significantly different between non-failing and failing cardiomyocytes. Therefore, the authors concluded that methylated CpG is relatively stable in chronic HF (8). However, this observation differs from other studies in which more differential DNA methylated regions and gene loci were identified. In fact, Meder et al. found 59 differential CpG regions in DCM comparing to non-failing hearts from a total of 72 left ventricular biopsies. Moreover, a recent study by Glezeva et al. using capture-based bisulfite sequencing method also identified 151 differential methylation regions in DCM in comparison with non-failing hearts (9). Such discrepant findings in DNA methylation patterns in the failing heart could be due to the different technologies for DNA methylation that were used in studies (whole genome bisulfite sequencing [WGBS] vs. bead array vs. capture-based bisulfite sequencing). Another reason could be that the study by Gilsbach et al. (8) used sorted cardiomyocytes whereas other studies used bulk left ventricle tissues (4,5,7,9), and Gilsbach's study only used 5 pairs of samples using WGBS method and the pooled biological replicates for DNA methylation analysis.

Taken together, all the published studies to date have demonstrated that alternation of DNA methylation is highly associated with DCM. Recently, coupling RNA-seq and genome-wide DNA methylation approaches, Pepin et al. linked DNA methylation to metabolic reprogramming in men with

end-stage ischemic cardiomyopathy (ICM). They also identified the differential DNA methylation between ICM and non-ischemic cardiomyopathy (NICM) (10). Specifically, the authors observed that 12.6% of CpG sites were differentially methylated between ICM and NICM, and that hypermethylation within promoter-associated CpG islands was observed in ICM samples. These hypermethylated gene loci are involved in oxidative metabolism and are downstream of an epigenetic repressor Enhancer of zeste homolog 2 (EZH2, a component of the polycomb repressive complex 2 and a histone methyltransferase) as well as Kuppel like factor 15 (KLF15), which is also an EZH2 target. These observations implied that human ischemic HF may display a distinctive DNA methylation signature associated with oxidative metabolism and anaerobic glycolysis. Furthermore, differential DNA methylation is likely mediated by EZH2-DNA methyltransferase complex to affect its downstream gene targets that are involved in metabolic reprogramming, such as KLF15.

Different etiologies of HF may generate different DNA methylation patterns. Using a targeted bisulfite sequence approach for patients with HF with various etiologies, Glezeva et al. identified 5 unique differentially methylated regions (DMR) in hypertrophic obstructive cardiomyopathy (HOCM), 151 DMRs in DCM and 55 DMRs in ICM and a total of the 209 genes were associated with these DMRs (9). Further validation for the genes associated with these DMRs confirmed that 6 protein-coding genes and 2 microRNA (miRNA) displayed significantly altered gene expression in at least 1 of the disease groups comparing with the control group. Among them, 2 novel HOCM-related genes were identified from this study, namely *HEY2* and *MSR1*. Both gene loci were hypermethylated in patients with HOCM, and their gene expression level was reduced. In addition, 2 genes involved in the regulation of the extracellular matrix, *CTGF* and *MMP2*, were found to be hypomethylated with increased gene expression level in DCM. Moreover, *COX17*, *MYMO3*, and miR24-1 were found to be hypermethylated with reduced gene expression, whereas *CTGF* miR155 was hypomethylated with elevated gene expression in ICM (9).

#### **POST-TRANSLATIONAL MODIFICATIONS OF HISTONES: DYNAMIC PROCESSES ACCOMPLISHED BY WRITERS AND READERS ENZYMATICALY**

Histone proteins are the component of the chromatin complex that forms chromosomes. The histone

octamer contains 2 copies of H2A, H2B, H3, and H4 proteins and forms the core of a nucleosome, wrapped 1.67 times by approximately 147 base pair (bp) of DNA. This conserved core complex is linked with a short linker DNA in a range between 20 and 80 bp and assembling into higher-order structures. The structures are stabilized by the linker histone H1, which usually binds to the entry/exit site of DNA on the surface of the nucleosomal core and wraps another 20 bp of DNA (11).

Unlike DNA methylation, in which the location of the methylation in the genome has a big impact on its functions, the regulatory function of the epigenetic modifications on histones are more delicate. The functions of histone modification are influenced not only by the location of these modifications (i.e., which amino acid residue and of genomic regions), but also by the type and the number of modifications on histones. The post-translational modifications (PTMs) of histones include acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation at different amino acid residues of the canonical histone proteins as well as variant histones such as H3.1, H3.3, H2A.Z, and macroH2A (12,13). These modifications affect the gene expression by changing chromatin structure to influence DNA accessibility or by recruiting various regulatory molecules such as histone modifiers, chromatin regulators, DNA repair molecules, and transcription factors. Studies from the past 2 decades have established a series of epigenetic codes for various histone modifications associated with the transcriptional status of genes. For example, acetylation of histone H3 at lysine 27 (K) residue marks active enhancers and promoter regions, whereas tri-methylation of histone H3 at K4 residues (H3K4me3) is associated with the active promoter region of genes. The comprehensive details of the histone codes associated with the regulation of gene expression have been reviewed elsewhere (12).

The PTMs of histones and their roles during cardiomyocyte differentiation and heart development for tissue regeneration have been studied extensively (14-18). However, our understanding of these regulations in HF remains unclear. The PTMs of histones is achieved by the actions of histone “writers” and “erasers.” They are enzymes that either add (write) PTM to histones or remove (erase) PTM from the histone proteins. Currently, the best understood the regulation of PTM on histones is on acetylation, methylation, phosphorylation (19). In this review, we will focus on 2 major PTMs to histone proteins - methylation and acetylation.

**HISTONE ACETYLATION AND DEACETYLATION.**

Acetylation commonly occurs on the lysine residues of the histone, which neutralizes the positive charge of the lysine and reduces the ionic interaction between histone proteins and DNA. This increases the DNA accessibility to transcription factors, chromatin remodelers, and modifiers and is frequently associated with transcription activation (20). Histone acetylation is controlled by histone acetyltransferase (HAT [writer]) and deacetylase (HDAC [eraser]). HATs can be divided into 2 different classes based on their localization in the cell (21). Type A HATs are in the nucleus playing an essential role in activation of gene transcription, whereas type B HATs are located in the cytoplasm mainly involved in the acetylation of newly synthesized histone. There are 4 major classes of HDAC classified by their catalytic domain (20,22). Class I HDACs, HDAC1, 2, 3, and 8, are widely expressed, whereas Class II (HDAC4, 5, 7, and 9 belong to the IIa group, and HDAC6 and 10 belong to the IIb group) are cell-type restricted. Class III HDACs are sirtuins 1-7 and they are NAD(+) dependent (20,22).

Evidence from animal models shows that HAT and HDAC are highly linked to cardiac hypertrophy. When CREB-binding protein/p300, a well-known HAT, was overexpressed in mouse myocardium, the mouse developed left ventricular myocyte hypertrophy, dilatation, and dysfunction (23). Cardiac-overexpression of p300 in mouse also acetylated GATA4, a zinc finger transcription factor known for mediating myocyte hypertrophy and increased DNA binding activity of GATA4 to its downstream target. This stimulated the expression of the GATA4-dependent hypertrophy-responsive genes including *Nppa*, prepro-endothelin-1, and  $\beta$ -myosin heavy chain (*Myh7*). In contrast, in inhibition of p300 activity with curcumin (a p300-specific HAT inhibitor) in a phenylephrine-induced hypertrophy rat model, it was observed that cardiomyocytes decreased acetylation of histone 3 and histone 4 as well as GATA4 DNA binding activity. In a hypertension salt-sensitive Dahl rat model as well as a surgically induced myocardial infarction (MI) rat model, oral curcumin administration prevented deterioration of systolic function and HF (24). Therefore, deacetylation or inhibition of HAT may play a role in HF.

Consistent with this notion, several studies have manipulated the expression of histone deacetylase in mouse hearts and in primary cardiomyocytes. Single knockout for deacetylase class II members (HDAC II) *Hdac5* or 9 in mice were sensitive to cardiac stress signals, such as pressure overload and calcineurin stimulation, and developed age-dependent cardiac hypertrophy (25,26). Moreover, expression of cardiac

hypertrophic genes (such as *Nppa* and *Myh7*) was enhanced in response to the stress signals in *Hdac5*- or *Hdac9*-null mice. This cardiac phenotype was caused by the activation of a prohypertrophic transcription factor, myocyte enhancer factor-2 (MEF2C), which can be inhibited by HDAC II (27). When infecting with a mutant form of *Hdac9* or *Hdac5* with more potent repressive activity on gene expression in phenylephrine-induced hypertrophic cardiomyocytes, it was demonstrated using chromatin immunoprecipitation coupled with quantitative PCR (ChIP-qPCR) assay that the acetylation state of histone 3 was significantly reduced at promoter regions of *Nppa* and *Myh7*. However, the gene loci that were not regulated during hypertrophy (e.g., *Gapdh*) were not affected. Furthermore, reduction of the acetylation on histone 3 correlated with the gene expression of *Nppa* and *Myh7* but not *Gapdh* (25). The results indicated that HDAC II has an inhibition role for cardiac hypertrophy.

Interestingly, when the activity of HDAC class I (HDAC I) was abolished, it had effects opposite to that of HDAC II. The inactivation for HDAC I member, *Hdac2*, in mice genetically or chemically, resulted in less sensitivity to hypertrophic stimuli, whereas the mice with *Hdac2* overexpression developed cardiac hypertrophy (28). The expression of cardiac hypertrophy genes, namely, *Nppa*, *Mhy7*, and *Acta1*, was not significantly different between control littermate and *Hdac2*<sup>-/-</sup> mice following transverse aortic constriction (TAC) or isoproterenol infusion (28). However, the state of histone acetylation was not investigated in this study. Cardiac-specific inactivation of *Hdac3*, another member of HDAC I, in mice caused cardiac hypertrophy as well as affected the genes associated with the fatty acid metabolism. Although there are no global changes of histone acetylation in *Hdac3* conditional knockout (cKO) hearts, histone acetylation was found to be increased at the promoter region of myocardial energetic genes and glucose use such as *Ucp2* and 3 (uncoupling protein 2 and 3), *fatty acyl-CoA synthetase*, *fatty acid transport protein*, and *Pyruvate dehydrogenase kinase 4* (29). These data suggested that HDAC3 may have a complementary role in regulating cardiac growth and myocardial energy metabolism (29).

**HISTONE METHYLATION AND DEMETHYLATION.**

Histone methylations can be associated with gene activation or repression, depending on which residue on histone the modification occurred. For example, the H3K4me3 is usually associated with active promoter activity whereas the tri-methylation of histone 3 lysine 9 (H3K9me3) is usually linked to

transcriptional repression (12,14). Methylation of histones also plays a critical role in the progress of HF as evidenced by the different effects in modulating of histone demethylase (HDM,[eraser]), co-factor or histone methyltransferase (HMT,[writer]) in cardiomyopathy mouse models. Zhang et al. demonstrated that JMJD2A, a HDM, promotes cardiac hypertrophy in TAC mice (30). Inactivation of *Jmjd2a* in the mouse myocardium resulted in an attenuated hypertrophic phenotype after the TAC procedure, whereas the overexpression of *Jmjd2a* increased the hypertrophic response to TAC-induced hypertrophy. This increasing development of hypertrophy is due to the activation of four-and-a-half LIM domain1 (FHL1), which is a key molecule in developing hypertrophy in the TAC mouse model (31). It was found that JMJD2A binds to the promoter region of FHL1 and reduced the level of H3K9me3 in the heart after the TAC procedure. Interestingly, JMJD2A was significantly up-regulated in patients with HOCM (30).

Another example of histone methylation can be found in disruption of dystrophin-glycoprotein complex in DCM. When a disruptor of telomeric silencing (*DOT1l*), the H3K79 methyltransferase, was specifically deleted in the mouse heart, mutant mice developed various abnormalities leading to cardiac remodeling and increased cardiomyocyte cell death (32). Further analysis of these mice found that the cardiac abnormalities were due to dysregulation of dystrophin (*Dmd*) in hearts. Consistent with its methyltransferase function, DOT1L was found to be directly bound to the *Dmd* gene locus in the postnatal mouse heart. This binding correlated positively with the enrichment of H3K79me2/3 in the *Dmd* gene body, whereas such enrichment was abolished in the *Dot1l* cKO mouse hearts (32).

Moreover, a recent study showed that G9a/EHMT2, another HMT, was found to be required for cardiomyocyte homeostasis to silence the fetal gene program in the adult heart (33). Conditional aberration of *G9a* in 2-month-old mouse myocardia caused various cardiac dysfunctions within the first 4 weeks of knockout induction. The results indicate that *G9a* is required for the proper cardiac function in normal mouse hearts. Further investigation of *G9a*-KO mice in comparison with control showed that *G9a* suppresses the key cardiac regulatory genes via H3K9me2. *G9a* also interacts with EZH2 (an HMT) and forms a complex with MEF2C to regulate MEF2C-dependent gene expression. Interestingly, *G9a* was found to be up-regulated during the initial stages of cardiac hypertrophy. Furthermore, chemical inactivation of *G9a* in TAC mice improved cardiac function and prevented the development of hypertrophy

suppressing the expression of anti-hypertrophy genes in cardiomyocytes (33). Taken together, *G9a* functions as an anti-hypertrophic regulator in healthy hearts, while serving as a hypertrophy activator in stressed hearts to promote cardiac hypertrophy.

Finally, the misregulation of the cofactor of histone methylation also showed effects on the regulation of HF. In vivo cardiac-specific deletion of the PAX transcription activation domain interacting protein (PTIP), a cofactor of H3K4 methylation, in adult mice caused the reduction of H3K4me3 (34). Using RNA microarray analysis, it was shown that a total of 221 genes were significantly altered in the *Ptip* cKO mouse hearts. The altered genes include Kv channel-interacting protein 2 (*Kcnip2*), a regulator for cardiac repolarization current that was previously shown to be down-regulated in a failing heart. Further analysis using ChIP-qPCR to detect H3K4me3 enrichment at 5' regulatory region of *Kcnip2* in control and *Ptip* cKO mouse hearts confirmed that the *Ptip* deletion directly affects the reduction of H3K4me3 at *Kcnip2* gene region (34). These findings support the notion that the methylation of histones plays a crucial role in modulating cardiac hypertrophy and HF.

One interesting study done by Hohl et al. showed that, although HDAC4 belongs to histone deacetylase, HDAC4 may play a central role for rapid modifications of H3K9 methylation in response to cardiac load. Using human left ventricular tissues in ChIP-qPCR assays, Hohl et al. found that the promoter regions of *Nppa* and *Nppb*, 2 hallmark genes for maladaptive remodeling of the left ventricle, displayed a higher level of activation marks for H3K9ac and H3K27ac in failing hearts, whereas the repressive marks, H3K9me3 and H3K9me2, were substantially reduced (35). Using a similar method, the authors also observed that heterochromatin protein 1 (HP1), a transcriptional repressor and H3K9me3 binding protein, was dissociated from the promoter regions, which may activate both *Nppa* and *Nppb* gene expression. The H3K9 demethylation and up-regulation of *NPPA* and *NPPB* in human failing myocardium correlated with the nuclear export of phosphorylated HDAC4. Inactivation of HDAC4 in the mouse hearts resulted in a reversal of these events in cardiomyocytes (35). How does a histone deacetylase "promote" demethylation of histone? Apparently 2 HMTs, JMJD1A and 2A, were up-regulated in failing human hearts. The up-regulation of *JMJD1A* was positively correlated with the increased *NPPA* and *NPPB* expression. In addition, both demethylases bind to the promoter region of *NPPA*. HDAC4 is associated with the transcriptional repression complex with the H3K9me3 binding protein, HP1, and the

histone methyltransferase, SUV39H. This complex is dissociated in response to the stress condition by the calmodulin-dependent protein kinase II  $\delta$ B-induced phosphorylation and nuclear export of HDCA4. Therefore, it is conceivable that the dissociation of the HDAC4-HP1-SUV39H complex opens up space at the promoter region of *Nppa* and *Nppb*, allowing the entry of the HMTs, JMJD1 and 2, to achieve the demethylation machinery in failing myocardium (35).

#### GENOME-WIDE STUDIES ON PTMs OF HISTONES.

Several genome-wide studies on PTMs of histone in myocardium have been conducted in animal models and humans. The first genome-wide study on methylation of histone in both human and rodent myocardium was published in 2009. Using a chromatin immunoprecipitates using genomic tiling arrays (ChIP-on-ChIP) technique, Kaneda et al. showed that the distribution patterns of H3K4me3 and H3K9me3 were significantly different between healthy and failing hearts (36). The differential distribution patterns of the 2 histone marks were found mainly at the gene loci involved in calcium signaling and cardiac contractility during the development of HF. In 2011, Movassagh et al. demonstrated that H3K36me3, a histone marker for active transcription, exhibited a differential methylation pattern and enrichment in cardiomyopathic and normal human hearts. The differential displays of H3K36me3 were mainly found in non-coding RNA loci, suggesting the involvement of the non-coding RNA in cardiomyopathy (4).

The most comprehensive genome-wide studies on PTMs of histones in cardiac hypertrophy and HF in an animal model was published in 2013 by Papait et al. Using ChIP followed by sequencing analysis for mouse cardiomyocytes isolated from sham and TAC mice for 7 histone epigenetic marks, Papait et al. identified that the level of histone acetylations (e.g., H3K9ac and H3K27ac) was decreased around the gene loci whose expression was down-regulated in TAC mice (37). In addition, there was a correlation between the gene expression level for those differentially expressed in TAC mice and the enrichment of histone methylation repression marks (e.g., H3K27me3, H3K9me2, and H3K9me3). The more active the transcription, the less enrichment for histone repression marks they observed (37). The authors also identified 9,207 cardiac-hypertrophy-specific-active enhancers that have DNA-binding motifs for MEF2C and its closely related family member, MEF2A. These results were validated using ChIP-qPCR analysis. The data suggested that MEF2A and 2C stimulate the cardiac hypertrophy gene expression via enhancers (37).

Although several genome-wide studies in the myocardium in both animals and humans have been published, their measurements were performed in bulk tissue specimens rather than in microdissected cells. Therefore, the data interpretation was based on several mixed-cell populations. Because epigenetic regulation is critical for cell-fate specification and differentiation, the effect of such regulation is highly cell-type-specific. Thus, it is necessary to determine the epigenetic signatures in a specific cell type. Recently, Gilsbach et al. used a nuclear staining with fluorescence-assisted sorting method to specifically isolate human cardiomyocytes from normal fetal, infant, and adult hearts as well as failing ones for a genome-wide epigenetic study (8). They showed that the whole-transcriptome regulation for normal cardiomyocytes during normal development is established by dynamic methylated CpG and common histone signatures at distal *cis*-acting elements of genes and gene bodies. One interesting finding from this study is that the expression of failing myocardium-related genes is modulated by active enhancer histone marks but not by DNA methylation. This observation is different from what was previously reported, and needs to be validated in a larger cohort study. Furthermore, because a heart is made up of other cell types besides cardiomyocytes (such as cardiac fibroblasts), it is necessary to generate epigenetic maps for other heart cell types.

In summary, our understanding of histone modifications on adult and failing hearts may have improved compared with a decade ago. However, we still lack systematic studies for normal and failing hearts in humans. Moreover, the currently published histone modification human studies have focused mainly on DCM and predominantly in males. Different types of cardiomyopathy may have their own distinct regulatory mechanisms. Sexual dimorphism can also link to different genetic and epigenetic regulations (38).

#### ATP-DEPENDENT CHROMATIN CONFORMATION, REMODELING, AND HF

---

The mammalian chromatin architecture and organization are highly regulated. The dynamic modification of the epigenome, including post-translational modification on histone tails and DNA methylation, causes chromatin remodeling and re-organization. This is an important process for controlling cell-fate determination, differentiation, and organ development (14). The chromatin regulators, such as histone modifiers, co-factors, and transcriptional regulators, can recruit or be recruited by other chromatin

regulators and transcription factors to regulate gene expression. By recruiting all necessary regulators, the distal *cis*-acting elements, such as enhancers, can form chromatin loops to interact with the proximal promoter regions of genes, and thus initiate and enhance the transcriptional activity of genes with the basal transcriptional complex (e.g., RNA polymerase II).

The histone writers and erasers achieve the PTMs on histones. These PTMs serve as “marks” to be recognized and interpreted by “readers” that carry on the action for regulating gene expression. The role of readers in HF is just emerging. Studies on modulating the function of bromodomain (BET) proteins, an acetylated-lysine binding protein and an epigenetic reader, have suggested that chromatin structure and organization are important in regulating the kinetics of gene expression in failing hearts (39,40). The BET family comprises BRD2, BRD3, BRD4, and BRDT. Among them, BRD4 protein expression was increased during cardiac hypertrophy. Inhibition of BET activity with the small molecule JQ1, either in mice or primary cell culture, suppressed phenylephrine-mediated cellular hypertrophy and pathological gene induction. Spiltoir et al. showed that the hypertrophic stimuli stimulated recruitment of BRD4 to the transcriptional start site of the gene *Nppa*. Binding of BRD4 to the ANF transcription start site was associated with increased phosphorylation of local RNA polymerase II. Recently, Duan et al. showed that administration of the JQ1 has therapeutic effects in animal HF models (41). Using an unbiased ingenuity pathway analysis, the authors showed that BET inhibition preferentially suppressed innate inflammatory and profibrotic transcriptional networks, namely, Nuclear Factor  $\kappa$ B and Transforming Growth Factor- $\beta$  signalings. Taken together, these studies suggested that BET proteins can be good pharmacological targets for treating HF.

In addition to the histone writers and readers and co-activators mentioned above, ATP-dependent chromatin remodeling complexes (ADCRs) are another group of regulators that may play a critical role in chromatin remodeling. ADCRs contain an adenosine triphosphate (ATPase) subunit that belongs to the SNF2 superfamily of proteins. The complex uses ATP hydrolysis as an energy source to alter or disrupt the histone-DNA interaction. In mammals, 4 ADCR families (the SWI/SNF, ISWS, CHD, and INO80) have been identified (42). Among these families, the roles of the SWI/SNF group in cardiac development and hypertrophy have been studied extensively during the past decade (43-48). These studies have found that BAF complex, the

mammalian analog of the SWI/SNF complex, is usually abundant in the embryonic heart but down-regulated in adult myocardium. The complex modulates gene expression of an important and motor molecule of the heart, myosin heavy chain (MHC). Two isoforms of MHC,  $\alpha$ - and  $\beta$ -MHC, are specifically expressed in the mammalian hearts, and they are located on the same chromosome (49). Alpha-MHC has higher ATPase activity than  $\beta$ -MHC. Alpha-MHC is expressed in adult cardiomyocytes primarily, whereas  $\beta$ -MHC is expressed in embryonic cardiomyocytes.

How does the BAF complex modulate the expression of these 2 isoforms? Using various myocardial-specific deletion of *Brg1* mouse models at different developmental stages, it was shown that BRG1, an important ATPase subunit of the BAF complex, interacts with HDACs and poly ADP-ribose polymerase (PARPs), e.g., PARP1, to suppress the adult isoform of  $\alpha$ -MHC, encoded by *Myh6* gene, and activates the fetal isoform ( $\beta$ -MHC), encoded by *Myh7* gene, in the embryonic heart. Because the BAF complex was down-regulated in adult cardiomyocytes, the suppression of  $\alpha$ -MHC is removed. Therefore, the expression of the adult isoform of MHC ( $\alpha$ -MHC) is up-regulated (46). Interestingly, the down-regulation of *BRG1* gene expression at birth was also observed in normal human hearts. However, unlike in adult mouse hearts where the BRG1 was undetectable in cardiomyocytes, it remains detectable in human adult cardiomyocytes, suggesting the other mechanisms may be present in regulating the switch of fetal and adult MHC in humans (50). Nevertheless, in hypertrophic and failing hearts, the subunits of BAF complexes and its binding partners, HDACs and PARPs, were increased and the expression of fetal MHC was up-regulated, whereas the adult MHC was decreased (46).

Another mechanism for the inhibition of adult *Myh6* gene is that BRG1 recruited G9a, a HMT, and DNA methyltransferase (DNMT3) to the promoter region of *Myh6* in the adult hypertrophic and failing hearts. The recruitment of G9a/DNMT3 complex resulted in the deposition of the H3K9me3, a repressive chromatin mark, and CpG methylation at promoter region of *Myh6*, which may subsequently activate the inhibition machinery for *Myh6* gene (51). When *Brg1* was specifically deleted in mouse hearts, the cardiac hypertrophy was diminished in the TAC mice. Therefore, it is conceivable that the proper expression and maintenance of the state of adult MHC expression can be a promising approach for treating HF. Moreover, BRG1 can directly increase the expression of osteopontin, a pro-fibrotic factor



**TABLE 1 Summary of Chromatin Regulator in Cardiomyopathy**

Regulator	Action	Effect	Reference
CREB-binding protein (P300)	Acetylation of histone tails.	(1) Increasing acetylation of MEF2 and GATA4. (2) Cardiac hypertrophy regulation.	23,24
HDAC2	Deacetylation of histone tails.	(1) Overexpression of HDAC2 caused stimulation of the Akt/GlaxoSmithKline3b pathway. (2) Cardiac hypertrophy regulation.	28
HDAC3	Deacetylation of histone tails.	Regulation of cardiac growth and myocardial energy metabolism.	29
HDAC4	(1) Deacetylation of histone tails. (2) Control H3K9 demethylation and HP1 dissociation to the <i>NPPA</i> promoter in response to elevated preload.	Mediation of H3K9 methylation and HP1 dissociation to <i>NPPA</i> promoter.	35
HDAC5 and 9	Deacetylation of histone tails.	Inhibit the transcriptional activity of MEF2c and act as negative regulator of cardiac hypertrophy.	25,26
DOT1L	Methylation of H3K79me.	Reduction of DOT1L activity causes DCM.	117
JMJD2A	Demethylation of H3K9me3, H3K4me3, and H3K27me3.	Activate cardiac hypertrophy and alter cardiac gene expression.	30
PTIP	(1) Co-factor of H3K4 methylation (2) Regulates the expression of <i>Kcnp2</i> .	Misregulation of PTIP cause cardiac hypertrophy and failure.	34
G9a/EHMT2	Methylation of H3K9me2 and H3K27me3 (lesser extent).	(1) Maintain cardiomyocyte homeostasis and interact with MEF2C to silence the fetal gene program in the adult heart. (2) Promote cardiac hypertrophy in stressed hearts.	33
SWARCA4 (BRG1)	(1) Regulates PARP-1/HDAC. (2) Recruits G9a and DNMT3.	(1) Activation of Brg1 diminished cardiac hypertrophy in TAC mice. (2) Activates fetal MHC isoform expression and repress adult MHC isoform in the hypertrophic and failing adult heart.	46,51
CTCF	Regulating chromatin 3D structure.	Inactivation of CTCF caused HF.	60

3D = 3 dimensional; CTCF = CCCTC-binding factor; *DOT1L* = disruptor of telomeric silencing1 like histone lysine methyltransferase; H3K4me3 = tri-methylation of lysine 4 on histone 3; H3K9me3 = tri-methylation of lysine 9 on histone 3; HDAC = histone deacetylase; HF = heart failure; HP1 = heterochromatin protein 1; *Kcnp2* = Kv channel-interacting protein 2; MHC = myosin heavy chain; *NPPA* = natriuretic peptide A; PARP = poly ADP-ribose polymerase; PTIP = PAX transcription activation domain interacting protein; TAC = transverse aortic constriction.

induced cardiac fibrosis (52). Thus, it seems that BRG1 acts as a stress-activated chromatin remodeler that controls fetal reprogramming of cardiac genes, e.g., the switch of adult form, *Myh6*, to fetal gene, *Myh7*, and activates hypertrophy and HF (46).

Chromatin remodeling complexes can swap histone variants, such as H3.3 and H2A.Z, with the canonical histone proteins in the nucleosome. It is clear now the incorporation of histone variants is critical for regulating the chromatin structure and gene expression. For example, a SWI/SNF chromatin-remodeling complex, SWR1, can switch H2A with H2A.Z at specific genomic loci to maintain genome integrity and to facilitate initiation of transcription, whereas INO80 chromatin-remodeling enzyme can evict H2A.Z from nucleosome to stabilize the chromatin (53).

The role of histone variants in development and diseases has been described elsewhere (54-56). Nevertheless, the role of histone variants in HF remains largely unknown. It was reported that the histone variant H2A.Z is up-regulated in TAC mice hearts and knockdown H2A.Z with shRNA in cultured rat cardiomyocytes under mechanical hypertrophic growth conditions attenuated cardiac hypertrophy and downregulated growth-related genes (namely, cyclin-dependent kinase 7 and ribosomal S6) (57). When HIRA, a histone chaperone

protein responsible for incorporation of histone variant H3.3, was specifically deleted in the mouse heart, the heart developed fibrosis, cardiac hypertrophy, as well as abnormal cardiac function. In addition, the sarcolemmal integrity was compromised in the *Hira*-deleted mouse (58). The histone variant H3.3 was shown to be associated with gene bodies of active genes in non-pluripotent cells and enriched at both active and silence genes in embryonic stem cell and precursor cells (59). Misregulation of incorporation of H3.3 would alter the gene expression. Indeed the cardiac transcriptome of *Hira*-cko hearts was altered in comparison with the control hearts.

Chromatin loops participated in the same regulatory territory, forming a higher order of chromatin structure known as topologically associating domains (TAD). Recently, the importance of TAD in HF has been reported (60). Rosa-Garrido et al. inspected the chromatin configuration differences among cardiomyocytes isolated from 3 different groups of mice and their correlation to the gene expression using high-resolution chromatin mapping and RNAseq approaches. These 3 groups are as follows: (1) normal adult, (2) induced-cardiac hypertrophy by TAC, and (3) cardiac-specific deletion of CCCTC-binding factor (CTCF) (*Ctcf*-KO) mice. CTCF was previously shown to regulate chromatin three-dimensional (3D) structure

**TABLE 2 Potential Epigenetics Drugs for HF**

Chemical	Action	Cardiac Outcome	Current Clinical Application	Ref. #'s
5-aza	DNA MTi.	Improved cardiac function partially.	ALL; AML; sickle cells disease.	113-115
Apicidin	HDACi for class I subtypes 1, 2, and 3.	Decreased myocardial hypertrophy.	NR	109
Curcumin	HATi for p300.	Prevented deterioration of systolic function and HF.	OA; RA; diabetes.	24,110-112
Givinostat (ITF2357)	Pan-HDACi.	Decreased inflammatory response and angiogenic effects. Reduced EMT and cardiac fibrosis.	Duchenne muscular dystrophy; juvenile idiopathic arthritis; polycythemia vera; myelofibrosis.	104-107
JQ1	BET bromodomain inhibitor.	Suppressed cardiac hypertrophy and pathological cardiac remodeling. Improved cardio function in TAC mice.	Variety of cancers.	39-41
SK-7041	HDACi for class I subtypes 1 and 2.	Reduced myocardial hypertrophy.	NR.	108
Trichostatin A	HDACi for class I and II.	Suppressed cardiac hypertrophy and improve survival rate.	Variety of cancers.	99
Valproic acid	HDACi for class I and II.	Suppressed cardiac hypertrophy and improved survival rate.	Variety of cancers.	108
Vorinostat (SAHA)	HDACi for class I and II.	Reduced infarct size and improved cardiac function	Cutaneous T-cell lymphoma.	102,103

5-aza = 5-azacytidine; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; BET = bromodomain; EMT = epithelia-mesenchymal transition; HDACi = histone deacetylase inhibitor; HMTi = histone methyltransferase inhibitor; MTi = methylation inhibition; NR = no report; OA = osteoarthritis; RA = rheumatoid arthritis; SAHA = suberoylanilide hydroxamic acid. Other abbreviations as in Table 1.

(61,62). Interestingly, mice with the cardiac-specific deletion of *Ctcf* developed HF. Moreover, the expression of CTCF was reduced in human patients with a failing heart and was increased in patients whose hearts were assisted by a left ventricle assist device. Rosa-Garrido et al. also described the alterations of chromatin architecture in healthy and diseased cardiac myocytes in mice. Significant alterations of chromatin loops and the boundary strength of TADs were found in cardiomyocytes from both *Ctcf*-KO and TAC mice. The enhancers and promoters interaction for major cardiac-related loci was also altered in the cardiomyocytes from failing hearts. In addition, there was a positive correlation between the alteration of gene expression levels and changes in chromatin compartmentalization in *Ctcf*-KO and TAC mice. It remains unclear if the similar TADs and chromatin looping structures and alterations would exist in human patients with HF.

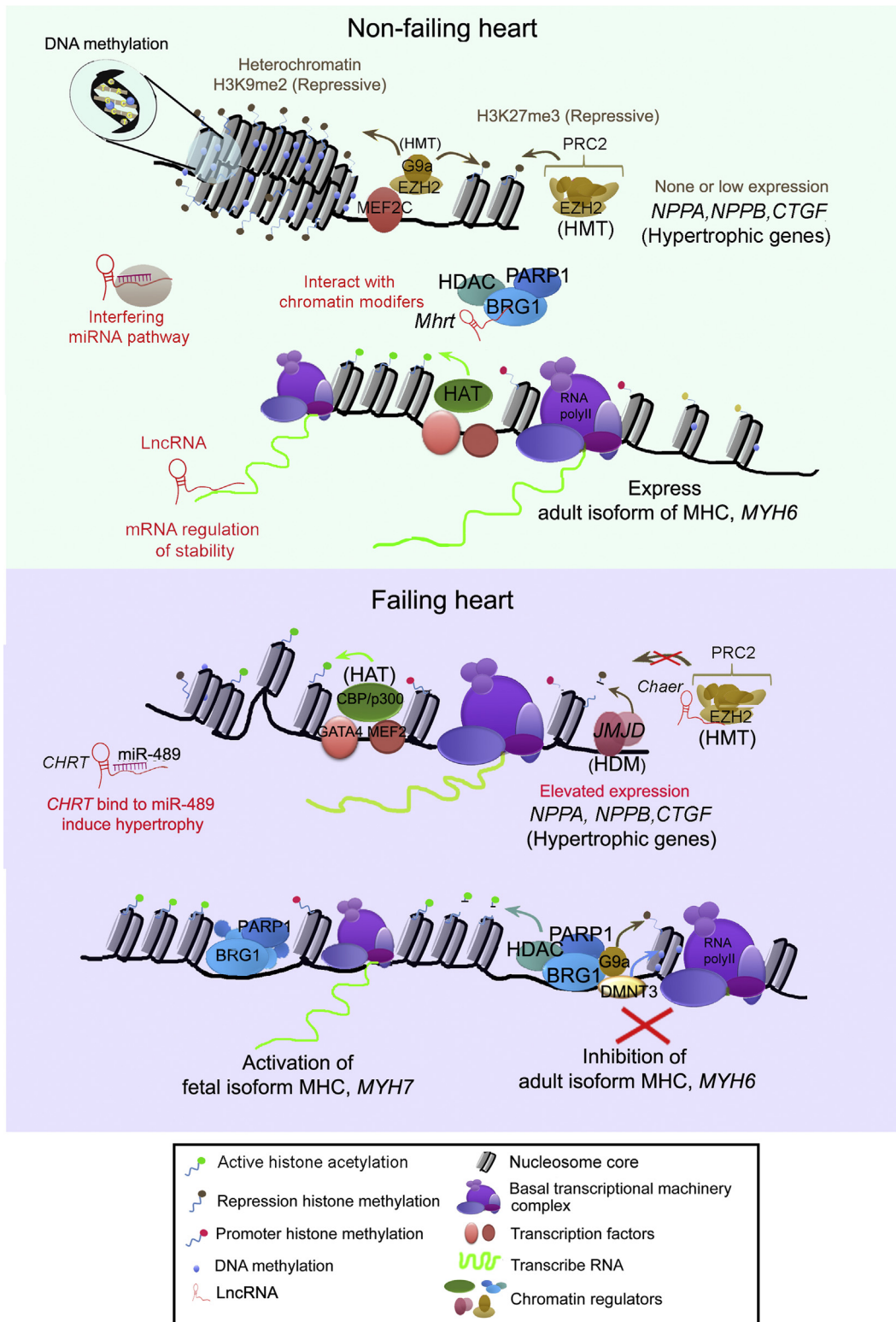
Using a chromosome conformation capture technique approach, Hi-C, to create the 3D map of the chromosome interaction for induced pluripotent stem cell-derived cardiomyocytes, Montefiori et al. recently linked the known cardiovascular disease-associated single nucleotide polymorphism to the target genes (63). The authors identified that the majority of non-coding regions could influence genes that are far away from them and some regions may modulate the gene expression for more than 2 genes. These findings are consistent with the notions that the long distance enhancers interact with the promoter to establish a long-range control of gene expression, and it is important to consider this aspect when interpreting functional targets of disease loci. A summary of epigenetic modifications and chromatin remodeling involved in cardiomyopathy is listed in Table 1.

### LONG NON-CODING RNAs: THE NEW PLAYERS IN EPIGENETIC REGULATION FOR HF

Only <2% of transcribed RNAs translate into proteins (64,65). Recent advances in transcriptomics and bioinformatics techniques revealed that untranslated RNAs (non-coding RNA [ncRNA]) are key regulators for transcription and translation in developmental as well as disease processes. In addition to the traditional, well-known non-coding RNAs (such as transfer RNA and ribosomal RNA), the rest of the non-coding RNAs can be classified into 2 categories based on their size. One category of ncRNA is the small nc-RNA, which is <200 nucleotides long. Small nc-RNA includes miRNA, piwi-interacting RNA, short interfering RNA, small nucleolar RNAs, small nuclear RNAs, extracellular RNAs, and small Cajal body-specific RNAs. The other category of ncRNA is the long ncRNAs (lncRNAs), which are longer than 200 nucleotides. lncRNAs have been linked to playing critical roles in chromatin remodeling. The epigenetic functions of small nc-RNA (especially miRNA) are extensive and have been reviewed elsewhere (66-70). Here we will focus on the epigenetic roles of lncRNA in HF.

Like other non-coding RNA, lncRNAs do not encode any protein product but elicit their functions via diverse mechanisms (71). For example, lncRNA can interact with chromatin regulators and transcriptional modifiers to enhance or repress expression of downstream genes. lncRNAs can also regulate gene expression by interfering with miRNA pathways. They also play essential roles on post-translational regulations that involve RNA splicing, messenger RNA (mRNA) stability, and translation. Furthermore, several lncRNAs have been identified to play vital roles during heart development (72).

**FIGURE 1** The Epigenetic Mechanisms in Heart Failure



Studies suggested that lncRNA plays a vital role in myocardium remodeling and pathogenesis in failing hearts. The mitochondria-derived lncRNA long intergenic non-coding RNA predicting cardiac remodeling (*LIPCAR*) was associated with left ventricular remodeling. This assumption is based on the observation that the *LIPCAR* is elevated in plasma of patients with HF after MI compared with MI patients without left ventricular remodeling (73). By lncRNA array screening, Piccoli et al. identified that cardiac fibroblast-specific lncRNA, maternally expressed gene 3, was the most abundant CF-lncRNA in the mice that underwent the TAC procedure during the first 4 weeks (74). The Wang and Xiao's group together identified that 15 and 135 lncRNAs were dysregulated in cardiac hypertrophy and failing mouse hearts, respectively (75). The study used whole-transcriptome analysis for left ventricle samples collected from mice after 1 week of TAC (hypertrophic stage), and 8 weeks of TAC procedure (end-stage HF). Among these dysregulated lncRNAs, 2 of them are particularly interesting. The first is *H19* and is a highly abundant and conserved imprinted lncRNA. *H19* was significantly up-regulated in HF samples in comparison with control samples. Consistent with this finding, *H19* was later shown to be a negative regulator of cardiomyocyte hypertrophy (76). Another interesting lncRNA from their findings is a cardiac hypertrophy-associated epigenetic regulator (*Chaer*), which is a heart-enriched lncRNA (77). *Chaer* was shown to be involved in cardiac hypertrophy development by directly binding to the catalytic subunit of polycomb repressor complex 2 (PRC2). PRC2 is responsible for methylation of H3K27me3 for gene silencing. The interaction between *Chaer* and PRC2 decreased the level of H3K27me3 in the

promoter region of cardiac hypertrophy-related genes, such as *NPPA*. Therefore, it may cause induction of these cardiac hypertrophy-related genes in hypertrophy hearts (77).

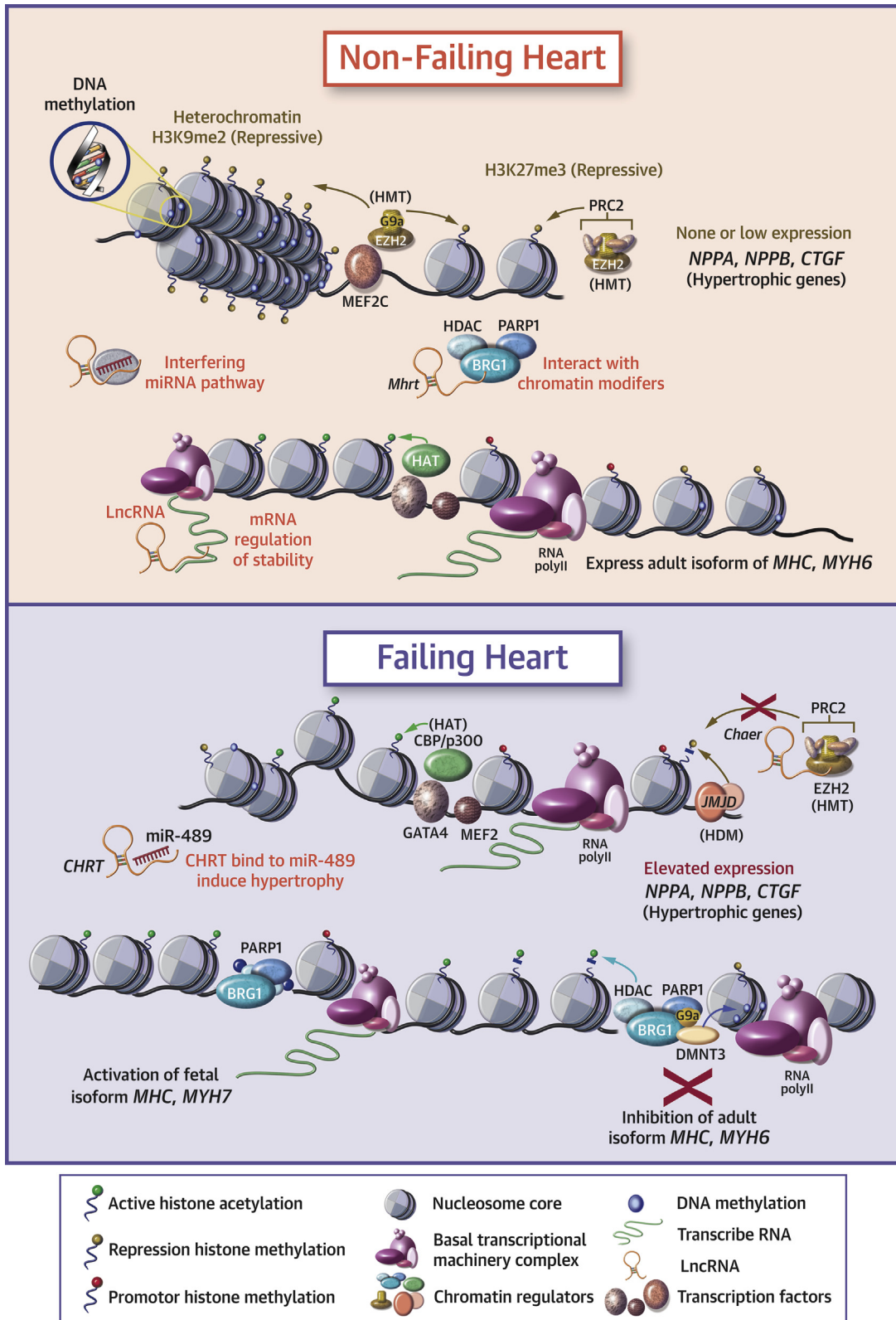
Long non-coding RNAs also regulate the chromatin-remodeling molecule to maintain cardiac functions. A cardiac-specific long-non RNA cluster, *Myheart* (*Mhrt*), was found to abound in the heart and have a protective role in pathological hypertrophy in heart (52). *Mhrt* is a cluster of lncRNA and was located at *myh6-myh7* gene loci. Expression of *Mhrt* was down-regulated in TAC mice hearts, in part due to the suppression by BRG1-HDAC-PARP chromatin remodeling complexes. Genetic restoration of *Mhrt779*, the most abundant *Mhrt* species, prevented the pathological hypertrophy phenotype after TAC in the heart. In fact, *Mhrt* can directly bind to the helicase domain of BRG1 that prevents the binding of BRG1 to its genomic DNA targets and thus prevents activation of downstream gene targets of BRG1, such as *Myh6* (52).

Using a global lncRNA expression profiling method, another cardio-specific enriched lncRNA, cardiac hypertrophy-associated transcript (*Chast*), was identified (78). *Chast* is specifically up-regulated in cardiomyocytes in TAC mice as well as in human patients who have aortic stenosis with hypertrophy heart phenotypes. Moreover, *Chast* seems to inhibit the expression of Pleckstrin homology domain-containing protein family member 1 (*Plekhm1*). *PLEKHM1* is an autophagy regulator. Because inhibition of autophagy in cardiomyocytes was shown to cause cardiac remodeling, it was proposed that *Chast* enhances cardiac hypertrophy and myocardium remodeling by inhibiting the function of *PLEKHM1* to prevent autophagy of cardiomyocytes.

**FIGURE 1 Continued**

Epigenetic regulations include DNA methylation, PTMs of histones, ATP-dependent chromatin conformation and remodeling, and non-coding RNA-mediated regulation. DNA methylated CpG-enriched region at the promoter region of genes is often associated with gene silencing, whereas methylated CpG found in the gene body is usually related to gene activation. Histone modifications associated with gene expression normally occur on histone 3. The histone writers (HAT and HMT) and erasers (HDAC and HMT) are responsible for PTMs of histone. The active histone mark, H3K27ac and repressive marks, H3K9me2 and H3K27me3, and promoter mark, H3K4me3, are shown. LncRNA can work with epigenetic regulators to effect activation or repression on chromosome remodeling and accessibility as well as the mRNA stability. In the non-failing heart, normal adult cardiac genes, adult isoform *MYH6*, express normally in cardiomyocyte. The cardiac hypertrophy genes, such as *NPPA* and *CTGF*, are not expressed or are at a basal level in non-failing hearts. The DNA around the promoter region of the hypertrophic-related gene loci is hypermethylated, and the chromatin state is inactive by marking H3K27me3 and/or H3K9me2. The PRC2/EZH2 and G9a-EZH2-MEF2C complexes involved in histone methylation in normal hearts are shown. It was shown that lncRNA, *Mhrt*, plays a critical role in maintaining *MYH6* expression in the adult heart by preventing BRG1 binding to the promoter region of *MYH6*. However, in failing hearts, in addition to normal cardiac gene expression, the hearts return to the fetal gene program and express the fetal isoform of myosin heavy chain, *MYH7*. This is likely due to the re-activation of BRG1 during heart failure. The potential mechanisms are shown. Two cardiac transcription factors, GATA4, MEF2C, and CBP/p300 (HAT), and JMJD (HMT) are involved in cardiac hypertrophy and heart failure. Two examples of the actions of lncRNAs, *CHRT* and *Chaer*, involved in cardiac hypertrophy and heart failure are presented. (Please see text for details.) ATP = adenosine triphosphate; CpG = cytosine-phosphate-guanine; HAT = histone acetyltransferase; HDAC = histone deacetylase; HMT = histone methyltransferase; mRNA = messenger RNA; *MYH6* = myosin heavy chain; *MYH7* = myosin heavy chain beta; PTM = post-translational modifications.

**CENTRAL ILLUSTRATION** The Epigenetic Mechanisms in Heart Failure



Using a deep sequencing of the non-coding RNA transcriptome method, Yang et al. found that the expression of lncRNAs, but not miRNA, is differentially expressed between nonischemic and ischemic human failing left ventricles (79). Interestingly, 10% of the differentially expressed lncRNAs can be improved or normalized for patients whose hearts were assisted with a left ventricular assist device. A recent study evaluated the potential of circulating lncRNAs as biomarkers for HF (80). Xuan et al. assessed the circulating levels of 13 known to be relevant to cardiovascular disease lncRNAs. Plasma samples from 72 patients with HF and 60 from non-HF patients were tested using quantitative reverse transcription-PCR. They identified 2 lncRNAs, NRON and MHRT, that may serve as novel predictive biomarkers for a failing heart.

As mentioned earlier, lncRNAs can also elicit their function by interfering with the function of miRNAs. One example is the interaction between lncRNA (cardiac hypertrophy-related factor [CHRF]) and *mir-489* (81). CHRF was shown to act as a sponge RNA to directly bind to *mir-489*, an anti-cardiac hypertrophy miRNA. This interaction affects the inhibition of myeloid differentiation primary response gene (*myd88*) by miR-489 and, therefore, causes cardiac hypertrophy.

### **NUTRITION, GUT MICROBIOME, AND EPIGENETICS: INTERPLAY AMONG HOST, GUT MICROBIOTIAL METABOLITES, AND HOST CHROMATIN**

In addition to the intrinsic factors, epigenetics is highly influenced by external factors, such as nutrients, toxins, stress, and other environmental factors directly or indirectly. Recently, attention has focused on how microbe diet interactions influence gene activity via epigenetic mechanisms that may affect an individual's health (82-84). Many gut metabolites, such as butyrate, acetate, and folate, may influence epigenetic processes by regulating enzyme activity that is responsible for epigenetic modifications (85). Butyrate, a short-chain fatty acid, is a potent inhibitor of HDACs and thus causes histone hyperacetylation (86). The acetylation of histone increases chromatin accessibility, which allows access of transcription factors and other critical factors to the open chromatin regions allowing for gene transcription. The presence of butyrate and its related sub-product, e.g., sodium butyrate, thus can alter the gene expression via inhibition of histone deacetylase (86-88). In colon cancer research, it was demonstrated that butyrate inhibits HDAC activity and

elicits its anti-inflammatory effects via suppressing the nuclear factor  $\kappa$ B and interferon  $\gamma$  production and increases the expression of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) (89,90). Many studies have shown that activation of the PPAR $\gamma$  has a beneficial role in cardiac diseases (91). Therefore, butyrate may have a direct protective role in cardiovascular disease via anti-inflammatory machinery. However, whether or not the anti-inflammatory effects of butyrate also involved epigenetic regulation in cardiovascular diseases remains elusive. In addition, Mathew et al. showed that the effect of butyrate on histone H3 modification altered the G1-specific cell cycle proteins and further resulted in the arrest of smooth muscle cell proliferation, which may provide an atheroprotective potential (92). The data suggested that butyrate may have a role in preventing cardiovascular diseases as well as in therapeutic intervention of arterial restenosis and in-stent-restenosis as a pharmacological agent, respectively (92).

Folate is a type of B vitamin that can be synthesized by gut bacteria or obtained from the diet, and is required for methionine homeostasis (85). It is a methyl donor to generate S-adenosylmethionine (SAM), which is a methyl-donating substrate for methyltransferase needed for the methylation of cytosine in DNA or lysine in histones. Folate and methionine deficiency leads to reduced SAM levels and subsequently reduces the methylation on histone 3 lysine (H3K4) in yeast and human cells as well as in an in vivo model (93,94). In addition, it was shown that the deficiency on dietary methyl-donors had a great impact on the epigenetic profile and also resulted in the development of metabolic diseases (95). However, the link between the gut microbiome-produced folate to HF remains unclear.

Choline is another methyl-group donor for epigenetic modification and is an essential nutrient. Choline is not only used by the host but also by the bacteria residing in the intestine of the host. Gut microbes used choline to produce trimethylamine (TMA), which can be further oxidized in the host's liver to generate trimethylamine N-oxide (TMAO), a metabolite linked to HF susceptibility, adverse prognosis, and induction of inflammation (96,97). By manipulating choline in a gnotobiotic mouse model harboring the synthetic gut bacterial community either with the 1 can consume choline and produce TMA (CC<sup>+</sup>) or the 1 can not (CC<sup>-</sup>), Romano et al. showed that gut bacteria compete with the host for the usage of choline. This competition caused the global reduction of DNA methylation on examined organs of the host, namely, brain, heart, liver, and colon (98). Despite, the elevated TMAO, behavior

changes, and exacerbation of metabolic disease, no cardiac phenotype was reported. Additionally, how TMAO influences the susceptibility of the failure and if this gut metabolite has any epigenetic role in the heart remain unclear. Although the ability of the gut microbiome to influence epigenetics has been established in cell lines and lower model organisms focusing on brain development, immune system, and cancer biology (86-88,92), data supporting their contributory role in HF and cardiovascular diseases are still emerging.

### THERAPEUTIC POTENTIAL OF TARGETING EPIGENETIC REGULATORS IN HF

Many drug candidates targeting epigenetic molecules have been identified for cancer therapy and other diseases. Also, several of them have been used in cardiac hypertrophy and failing heart studies in cellular and animal models (Table 2). Those epigenetic-candidate drugs may also be used to treat patients with cardiac hypertrophy and HF. One promising approach is targeting the BET complex. As mentioned earlier, BET proteins play roles in chromatin remodeling and establishing the basic transcriptional complex at the promoter region of genes to regulate gene expression. Several studies showed that administration of a BET inhibitor, JQ1, *in vivo* and *in vitro* could suppress pathological cardiac remodeling, suppress pathological hypertrophy, and block the innate inflammatory and profibrotic myocardial program (39,41).

Another potential target of epigenetic molecules is HDAC. There are many HDAC inhibitors (HDACi) and animal testing demonstrated the beneficial effects of HDACi treatments in preventing or improving cardiac functions. Treatment of either trichostatin A and scriptaid, 2 broad-spectrum HDACis, in a pressure-overload hypertrophy mouse model revealed that cardiac hypertrophy and fibrosis were suppressed, and the hypertrophy-associated switch of adult and fetal isoforms of myosin heavy chain expression was diminished (99). In addition, the same HDAC inhibitors were shown to reduce myocardial infarct size and to preserve systolic function in a Langendorff perfusion *in vitro* model as well as in a mouse ischemia/reperfusion model (100,101). Using mouse and rabbit ischemic/reperfusion models, Xie et al. showed that suberoylanilide hydroxamic acid (SAHA, also known as Vorinostat), a Food and Drug Administration-approved HDACi for cancer treatment (102), also can preserve the systolic function and reduce infarct size (103). Additionally, using a

simulated ischemic/reperfusion neonatal rat ventricular myocyte culture system, Xie et al. further demonstrated that the cardioprotective effects are partly due to induction of cardiomyocyte autophagy (103). Givinostat, another pan-HDACi, which is used in treating Duchenne muscular dystrophy, inflammatory diseases, myelofibrosis, and blood cancers (104-106), has been shown to attenuate inflammatory response and angiogenic effects, reduce endothelial-to-mesenchymal transition, and reduce cardiac fibrosis on an acute MI mouse model (107). Moreover, when using SK-7041, a Class I HDACi, in either angiotensin II infusion or TAC cardiac hypertrophy mouse models, Kee et al. demonstrated that myocardial hypertrophy was reduced and the survival rate was improved in mouse and rat hypertrophy models (108). Using an *in vitro* screening assay, Gallo et al. identified a truncated form of apicidin, another class I HDACi, and this HDACi also demonstrated similar results as what Kee et al. found (109). These results suggested that cardioprotective effects were mainly mediated by class I HDACs.

Many small molecules for inhibition of HDAC have been developed and tested to treat cardiac diseases. However, unlike HDAC, only a few inhibitors of HAT currently show a potential protective effect against cardiac disease. One of them is curcumin, which was first used as an anti-inflammatory reagent. Currently, curcumin has been suggested for treating osteoarthritis, rheumatoid arthritis, and diabetes (110-112). It is a natural compound derived from the active ingredient of turmeric (*Curcuma longa*), which can act as a p300-specific histone HAT inhibitor. Oral treatment with curcumin in a rat MI model demonstrated improvement in left ventricular function and reduction of fibrosis compared with non-treatment MI rats (24).

A DNA methylation inhibitor, 5-azacytidine (5-aza), is widely used to treat leukemia and sickle cell diseases (113,114). In a spontaneously hypertensive rat model, intraperitoneal injection of 5-aza at a dose of 10 mg/kg resulted in improvement in cardiac function as well as reduction in cardiac fibrosis (115).

Last but not least, lncRNA silencing approach using GapmeR, a type of antisense oligonucleotide, is another potential therapeutic target for HF (116). Although no clinical trials targeting lncRNAs were performed, several *in vivo* studies have shown that injection of lncRNA GapmeR such as GapmeR-*Chast* preserved or improved cardiac function in mice after pressure overload-induced cardiac hypertrophy and HF (74,78).

## CONCLUSIONS AND FUTURE PERSPECTIVES

This review summarizes recent studies that supported key contributing roles of epigenetic mechanisms in cardiac hypertrophy and failure and may have important therapeutic implications for HF (Figure 1, Central Illustration). However, the ubiquitous characteristics of epigenetic regulators have created a significant challenge to develop targeted epigenetic therapies for cardiac diseases. First, because most epigenetic regulators modulate gene expression via conventional mechanisms, the global inhibition or activation of such regulators may have pleiotropic effects with no cardiac specificity. Second, the heart is made up of several different types of cells. Each cell type may possess its own epigenetic signature in response to disease conditions. Before generalizing such a knowledge base for cardiac therapies, larger cohort studies with cell-specific epigenetic maps for various types of cardiomyopathy are necessary. Equally important is the need to identify cardiac disease-specific genes and their corresponding epigenetic regulators and to establish networks between the epigenetic alterations and the signaling transduction cascades (specifically to cardiac

hypertrophy and failing hearts). Another challenge of epigenetic therapy for HF (as is true for other diseases) is to minimize the off-target effects by developing suitable and efficient methods for delivering to the specific locations within a heart. Lastly, a potential near-term application of the current knowledge base of epigenetic regulation for cardiac diseases is the development of epigenetic biomarkers for diagnosis, prognosis, and therapy optimization, especially from easily accessible tissues (such as peripheral blood or blood cells) that may reflect cardiac-specific alterations. Generating such insights into epigenetic modifications along the natural history of human HF will catalyze the development of personalized therapies for cardiomyopathy and HF.

**ACKNOWLEDGMENTS** We thank Dr. Kymberleigh Romano and Ms. Kirsten Bede for review of the manuscript and suggestions.

**ADDRESS FOR CORRESPONDENCE:** Dr. W.H. Wilson Tang, Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic, 9500 Euclid Avenue, Desk J3-4, Cleveland, Ohio 44195. E-mail: [tangw@ccf.org](mailto:tangw@ccf.org).

## REFERENCES

1. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* 2012;13:484-92.
2. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* 1983;301:89-92.
3. Esteller M. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. *Oncogene* 2002;21:5427-40.
4. Movassagh M, Choy MK, Goddard M, Bennett MR, Down TA, Foo RS. Differential DNA methylation correlates with differential expression of angiogenic factors in human heart failure. *PLoS One* 2010;5:e8564.
5. Movassagh M, Choy MK, Knowles DA, et al. Distinct epigenomic features in end-stage failing human hearts. *Circulation* 2011;124:2411-22.
6. Haas J, Frese KS, Park YJ, et al. Alterations in cardiac DNA methylation in human dilated cardiomyopathy. *EMBO Mol Med* 2013;5:413-29.
7. Meder B, Haas J, Sedaghat-Hamedani F, et al. Epigenome-wide association study identifies cardiac gene patterning and a novel class of biomarkers for heart failure. *Circulation* 2017;136:1528-44.
8. Gilsbach R, Schwaderer M, Preissl S, et al. Distinct epigenetic programs regulate cardiac myocyte development and disease in the human heart in vivo. *Nat Commun* 2018;9:391.
9. Glezeva N, Moran B, Collier P, et al. Targeted DNA methylation profiling of human cardiac tissue reveals novel epigenetic traits and gene deregulation across different heart failure patient subtypes. *Circ Heart Fail* 2019;12:e005765.
10. Pepin ME, Ha CM, Crossman DK, et al. Genome-wide DNA methylation encodes cardiac transcriptional reprogramming in human ischemic heart failure. *Lab Invest* 2019;99:371-86.
11. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997;389:251-60.
12. Zhao Y, Garcia BA. Comprehensive catalog of currently documented histone modifications. *Cold Spring Harb Perspect Biol* 2015;7:a025064.
13. Bowman GD, Poirier MG. Post-translational modifications of histones that influence nucleosome dynamics. *Chem Rev* 2015;115:2274-95.
14. Chen T, Dent SY. Chromatin modifiers and remodellers: regulators of cellular differentiation. *Nat Rev Genet* 2014;15:93-106.
15. BurrIDGE PW, Sharma A, Wu JC. Genetic and epigenetic regulation of human cardiac reprogramming and differentiation in regenerative medicine. *Annu Rev Genet* 2015;49:461-84.
16. Preissl S, Schwaderer M, Raulf A, et al. Deciphering the epigenetic code of cardiac myocyte transcription. *Circ Res* 2015;117:413-23.
17. Schmitt AD, Hu M, Jung I, et al. A compendium of chromatin contact maps reveals spatially active regions in the human genome. *Cell Reports* 2016;17:2042-59.
18. Quaife-Ryan GA, Sim CB, Ziemann M, et al. Multicellular transcriptional analysis of mammalian heart regeneration. *Circulation* 2017;136:1123-39.
19. Rothbart SB, Strahl BD. Interpreting the language of histone and DNA modifications. *Biochim Biophys Acta* 2014;1839:627-43.
20. Gray SG, Teh BT. Histone acetylation/deacetylation and cancer: an "open" and "shut" case? *Curr Mol Med* 2001;1:401-29.
21. Lee KK, Workman JL. Histone acetyltransferase complexes: 1 size doesn't fit all. *Nat Rev Mol Cell Biol* 2007;8:284-95.
22. Marmorstein R. Structure of histone deacetylases: insights into substrate recognition and catalysis. *Structure* 2001;9:1127-33.
23. Yanazume T, Hasegawa K, Morimoto T, et al. Cardiac p300 is involved in myocyte growth with decompensated heart failure. *Mol Cell Biol* 2003;23:3593-606.
24. Morimoto T, Sunagawa Y, Kawamura T, et al. The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. *J Clin Invest* 2008;118:868-78.
25. Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act



- as signal-responsive repressors of cardiac hypertrophy. *Cell* 2002;110:479-88.
26. Chang S, McKinsey TA, Zhang CL, Richardson JA, Hill JA, Olson EN. Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. *Mol Cell Biol* 2004;24:8467-76.
  27. McKinsey TA, Zhang CL, Olson EN. MEF2: a calcium-dependent regulator of cell division, differentiation and death. *Trends Biochem Sci* 2002;27:40-7.
  28. Trivedi CM, Luo Y, Yin Z, et al. Hdac2 regulates the cardiac hypertrophic response by modulating Gsk3 beta activity. *Nat Med* 2007;13:324-31.
  29. Montgomery RL, Potthoff MJ, Haberland M, et al. Maintenance of cardiac energy metabolism by histone deacetylase 3 in mice. *J Clin Invest* 2008;118:3588-97.
  30. Zhang QJ, Chen HZ, Wang L, Liu DP, Hill JA, Liu ZP. The histone trimethyllysine demethylase JMJD2A promotes cardiac hypertrophy in response to hypertrophic stimuli in mice. *J Clin Invest* 2011;121:2447-56.
  31. Sheikh F, Raskin A, Chu PH, et al. An FHL1-containing complex within the cardiomyocyte sarcomere mediates hypertrophic biomechanical stress responses in mice. *J Clin Invest* 2008;118:3870-80.
  32. Nguyen AT, Xiao B, Neppel RL, et al. DOT1L regulates dystrophin expression and is critical for cardiac function. *Genes Dev* 2011;25:263-74.
  33. Papat R, Serio S, Pagiatakis C, et al. Histone methyltransferase G9a is required for cardiomyocyte homeostasis and hypertrophy. *Circulation* 2017;136:1233-46.
  34. Stein AB, Jones TA, Herron TJ, et al. Loss of H3K4 methylation destabilizes gene expression patterns and physiological functions in adult murine cardiomyocytes. *J Clin Invest* 2011;121:2641-50.
  35. Hohl M, Wagner M, Reil JC, et al. HDAC4 controls histone methylation in response to elevated cardiac load. *J Clin Invest* 2013;123:1359-70.
  36. Kaneda R, Takada S, Yamashita Y, et al. Genome-wide histone methylation profile for heart failure. *Genes Cells* 2009;14:69-77.
  37. Papat R, Cattaneo P, Kunderfranco P, et al. Genome-wide analysis of histone marks identifying an epigenetic signature of promoters and enhancers underlying cardiac hypertrophy. *Proc Natl Acad Sci U S A* 2013;110:20164-9.
  38. Ratnu VS, Emami MR, Bredy TW. Genetic and epigenetic factors underlying sex differences in the regulation of gene expression in the brain. *J Neurosci Res* 2017;95:301-10.
  39. Anand P, Brown JD, Lin CY, et al. BET bromodomains mediate transcriptional pause release in heart failure. *Cell* 2013;154:569-82.
  40. Spiltoir JI, Stratton MS, Cavin MA, et al. BET acetyl-lysine binding proteins control pathological cardiac hypertrophy. *J Mol Cell Cardiol* 2013;63:175-9.
  41. Duan Q, McMahon S, Anand P, et al. BET bromodomain inhibition suppresses innate inflammatory and profibrotic transcriptional networks in heart failure. *Sci Transl Med* 2017;9:pii: eaah5084.
  42. Narlikar GJ, Sundaramoorthy R, Owen-Hughes T. Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. *Cell* 2013;154:490-503.
  43. Banerjee R, Bultman SJ, Holley D, et al. Non-targeted metabolomics of Brg1/Brm double-mutant cardiomyocytes reveals a novel role for SWI/SNF complexes in metabolic homeostasis. *Metabolomics* 2015;11:1287-301.
  44. Bevilacqua A, Willis MS, Bultman SJ. SWI/SNF chromatin-remodeling complexes in cardiovascular development and disease. *Cardiovasc Pathol* 2014;23:85-91.
  45. Bultman SJ, Holley DW, G GdR, et al. BRG1 and BRM SWI/SNF ATPases redundantly maintain cardiomyocyte homeostasis by regulating cardiomyocyte mitophagy and mitochondrial dynamics in vivo. *Cardiovasc Pathol* 2016;25:258-69.
  46. Hang CT, Yang J, Han P, et al. Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature* 2010;466:62-7.
  47. Scott CA, Marsden AN, Rebagliati MR, et al. Nuclear/cytoplasmic transport defects in BBS5 underlie congenital heart disease through perturbation of a chromatin remodeling protein. *PLoS genetics* 2017;13:e1006936.
  48. Vieira JM, Howard S, Villa Del Campo C, et al. BRG1-SWI/SNF-dependent regulation of the Wt1 transcriptional landscape mediates epicardial activity during heart development and disease. *Nat Commun* 2017;8:16034.
  49. England J, Loughna S. Heavy and light roles: myosin in the morphogenesis of the heart. *Cell Mol Life Sci* 2013;70:1221-39.
  50. Qian Y, Xiao D, Guo X, et al. Hypomethylation and decreased expression of BRG1 in the myocardium of patients with congenital heart disease. *Birth Defects Res* 2017;109:1183-95.
  51. Han P, Li W, Yang J, et al. Epigenetic response to environmental stress: assembly of BRG1-G9a/GLP-DNMT3 repressive chromatin complex on Myh6 promoter in pathologically stressed hearts. *Biochim Biophys Acta* 2016;1863:1772-81.
  52. Han P, Li W, Lin CH, et al. A long noncoding RNA protects the heart from pathological hypertrophy. *Nature* 2014;514:102-6.
  53. Billon P, Cote J. Precise deposition of histone H2A.Z in chromatin for genome expression and maintenance. *Biochim Biophys Acta* 2013;1819:290-302.
  54. Chen P, Zhao J, Li G. Histone variants in development and diseases. *J Genet Genomics* 2013;40:355-65.
  55. Gaume X, Torres-Padilla ME. Regulation of reprogramming and cellular plasticity through histone exchange and histone variant incorporation. *Cold Spring Harb Symp Quant Biol* 2015;80:165-75.
  56. Yuen BT, Knoepfler PS. Histone H3.3 mutations: a variant path to cancer. *Cancer Cell* 2013;24:567-74.
  57. Chen IY, Lypowy J, Pain J, et al. Histone H2A.z is essential for cardiac myocyte hypertrophy but opposed by silent information regulator 2alpha. *J Biol Chem* 2006;281:19369-77.
  58. Valenzuela N, Fan Q, Fa'ak F, et al. Cardiomyocyte-specific conditional knockout of the histone chaperone HIRA in mice results in hypertrophy, sarcolemmal damage and focal replacement fibrosis. *Dis Model Mech* 2016;9:335-45.
  59. Ahmad K, Henikoff S. The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol Cell* 2002;9:1191-200.
  60. Rosa-Garrido M, Chapski DJ, Schmitt AD, et al. High-resolution mapping of chromatin conformation in cardiac myocytes reveals structural remodeling of the epigenome in heart failure. *Circulation* 2017;136:1613-25.
  61. Filippova GN. Genetics and epigenetics of the multifunctional protein CTCF. *Curr Top Dev Biol* 2008;80:337-60.
  62. Garrick D, De Gobbi M, Gibbons R, Higgs DR. CTCF, cohesin and higher-order chromatin structure. *Epigenomics* 2009;1:232.
  63. Montefiori LE, Sobreira DR, Sakabe NJ, et al. A promoter interaction map for cardiovascular disease genetics. *Elife* 2018;7:pii: e35788.
  64. Project E. The ENCODE (ENCyclopedia Of DNA Elements) Project. *Science* 2004;306:636-40.
  65. Hangauer MJ, Vaughn IW, McManus MT. Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. *PLoS Genetics* 2013;9:e1003569.
  66. de Lucia C, Komici K, Borghetti G, et al. microRNA in cardiovascular aging and age-related cardiovascular diseases. *Front Med* 2017;4:74.
  67. Shah P, Bristow MR, Port JD. MicroRNAs in heart failure, cardiac transplantation, and myocardial recovery: biomarkers with therapeutic potential. *Curr Heart Failure Reports* 2017;14:454-64.
  68. Wojciechowska A, Braniewska A, Kozar-Kaminska K. MicroRNA in cardiovascular biology and disease. *Adv Clin Experimental Med* 2017;26:865-74.
  69. Bayoumi AS, Aonuma T, Teoh JP, Tang YL, Kim IM. Circular noncoding RNAs as potential therapies and circulating biomarkers for cardiovascular diseases. *Acta Pharmacologica Sinica* 2018;7:1100-9.
  70. Mellis D, Caporali A. MicroRNA-based therapeutics in cardiovascular disease: screening and delivery to the target. *Biochem Soc Trans* 2018;46:11-21.
  71. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet* 2014;15:7-21.
  72. Hennessy EJ. Cardiovascular disease and long noncoding RNAs: tools for unraveling the mystery Lnc-ing RNA and phenotype. *Circ Cardiovasc Genet* 2017;10:e001556.
  73. Kumarswamy R, Bauters C, Volkman I, et al. Circulating long noncoding RNA, LIPCAR, predicts

- survival in patients with heart failure. *Circ Res* 2014;114:1569-75.
74. Piccoli MT, Gupta SK, Viereck J, et al. Inhibition of the cardiac fibroblast-enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction. *Circ Res* 2017;121:575-83.
75. Lee JH, Gao C, Peng G, et al. Analysis of transcriptome complexity through RNA sequencing in normal and failing murine hearts. *Circ Res* 2011;109:1332-41.
76. Liu L, An X, Li Z, et al. The H19 long noncoding RNA is a novel negative regulator of cardiomyocyte hypertrophy. *Cardiovasc Res* 2016;111:56-65.
77. Wang Z, Zhang XJ, Ji YX, et al. The long noncoding RNA Chaer defines an epigenetic checkpoint in cardiac hypertrophy. *Nat Med* 2016;22:1131-9.
78. Viereck J, Kumarswamy R, Foinquinos A, et al. Long noncoding RNA Chast promotes cardiac remodeling. *Sci Transl Med* 2016;8:326ra22.
79. Yang KC, Yamada KA, Patel AY, et al. Deep RNA sequencing reveals dynamic regulation of myocardial noncoding RNAs in failing human heart and remodeling with mechanical circulatory support. *Circulation* 2014;129:1009-21.
80. Xuan L, Sun L, Zhang Y, et al. Circulating long non-coding RNAs NRON and MHRT as novel predictive biomarkers of heart failure. *J Cell Mol Med* 2017;21:1803-14.
81. Wang K, Liu F, Zhou LY, et al. The long non-coding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. *Circ Res* 2014;114:1377-88.
82. Sanders LM, Zeisel SH. Choline: dietary requirements and role in brain development. *Nutr Today* 2007;42:181-6.
83. Sezgin Z, Dincer Y. Alzheimer's disease and epigenetic diet. *Neurochem Int* 2014;78:105-16.
84. Aleksandrova K, Romero-Mosquera B, Hernandez V. Diet, gut microbiome and epigenetics: emerging links with inflammatory bowel diseases and prospects for management and prevention. *Nutrients* 2017;9. pii:E962.
85. Jeffery IB, O'Toole PW. Diet-microbiota interactions and their implications for healthy living. *Nutrients* 2013;5:234-52.
86. Candido EP, Reeves R, Davie JR. Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell* 1978;14:105-13.
87. Davie JR. Inhibition of histone deacetylase activity by butyrate. *J Nutr* 2003;133:2485S-93S.
88. Aoyama M, Kotani J, Usami M. Butyrate and propionate induced activated or non-activated neutrophil apoptosis via HDAC inhibitor activity but without activating GPR-41/GPR-43 pathways. *Nutrition* 2010;26:653-61.
89. Andoh A, Fujiyama Y, Hata K, et al. Counter-regulatory effect of sodium butyrate on tumour necrosis factor- $\alpha$  (TNF- $\alpha$ )-induced complement C3 and factor B biosynthesis in human intestinal epithelial cells. *Clin Exp Immunol* 1999; 118:23-9.
90. Place RF, Noonan EJ, Giardina C. HDAC inhibition prevents NF- $\kappa$ B activation by suppressing proteasome activity: down-regulation of proteasome subunit expression stabilizes I  $\kappa$ B  $\alpha$ . *Biochem Pharmacol* 2005;70:394-406.
91. Khuchua Z, Glukhov AI, Strauss AW, Javadov S. Elucidating the beneficial role of PPAR agonists in cardiac diseases. *Int J Mol Sci* 2018;19(11):pii: E3464.
92. Mathew OP, Ranganna K, Yatsu FM. Butyrate, an HDAC inhibitor, stimulates interplay between different posttranslational modifications of histone H3 and differently alters G1-specific cell cycle proteins in vascular smooth muscle cells. *Biomed Pharmacother* 2010;64:733-40.
93. Sadhu MJ, Guan Q, Li F, et al. Nutritional control of epigenetic processes in yeast and human cells. *Genetics* 2013;195:831-44.
94. Mentch SJ, Mehrmohamadi M, Huang L, et al. Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. *Cell Metab* 2015;22:861-73.
95. Obeid R. The metabolic burden of methyl donor deficiency with focus on the betaine homocysteine methyltransferase pathway. *Nutrients* 2013;5:3481-95.
96. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;472:57-63.
97. Organ CL, Otsuka H, Bhushan S, et al. Choline diet and its gut microbe-derived metabolite, trimethylamine N-Oxide, exacerbate pressure overload-induced heart failure. *Circ Heart Fail* 2016;9:e002314.
98. Romano KA, Martinez-Del Campo A, Kasahara K, et al. Metabolic, epigenetic, and transgenerational effects of gut bacterial choline consumption. *Cell Host Microbe* 2017;22:279-90 e7.
99. Kong Y, Tannous P, Lu G, et al. Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation* 2006; 113:2579-88.
100. Granger A, Abdullah I, Huebner F, et al. Histone deacetylase inhibition reduces myocardial ischemia-reperfusion injury in mice. *FASEB J* 2008;22:3549-60.
101. Zhao TC, Cheng G, Zhang LX, Tseng YT, Padbury JF. Inhibition of histone deacetylases triggers pharmacologic preconditioning effects against myocardial ischemic injury. *Cardiovasc Res* 2007;76:473-81.
102. Bubna AK. Vorinostat-an overview. *Indian J Dermatol* 2015;60:419.
103. Xie M, Kong Y, Tan W, et al. Histone deacetylase inhibition blunts ischemia/reperfusion injury by inducing cardiomyocyte autophagy. *Circulation* 2014;129:1139-51.
104. Bose P, Verstovsek S. Developmental therapeutics in myeloproliferative neoplasms. *Clin Lymphoma Myeloma Leukemia* 2017;17s:S43-52.
105. Mauro A, Rigante D, Cimaz R. Investigational drugs for treatment of juvenile idiopathic arthritis. *Expert Opin Invest Drugs* 2017;26:381-7.
106. Shawi F, Perras C, Severn M. Emerging drugs for Duchenne Muscular Dystrophy. *CADTH Issues in Emerging Health Technologies*. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health, CADTH 2017. , 2016:1-19.
107. Milan M, Pace V, Maiullari F, et al. Givinostat reduces adverse cardiac remodeling through regulating fibroblasts activation. *Cell Death Dis* 2018;9:108.
108. Kee HJ, Sohn IS, Nam KI, et al. Inhibition of histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding. *Circulation* 2006;113:51-9.
109. Gallo P, Latronico MV, Gallo P, et al. Inhibition of class I histone deacetylase with an apicidin derivative prevents cardiac hypertrophy and failure. *Cardiovasc Res* 2008;80:416-24.
110. Amalraj A, Varma K, Jacob J, et al. A novel highly bioavailable curcumin formulation improves symptoms and diagnostic indicators in rheumatoid arthritis patients: a randomized, double-blind, placebo-controlled, two-dose, three-arm, and parallel-group study. *J Med Food* 2017;20:1022-30.
111. Haroyan A, Mukuchyan V, Mkrtchyan N, et al. Efficacy and safety of curcumin and its combination with boswellic acid in osteoarthritis: a comparative, randomized, double-blind, placebo-controlled study. *Bmc Complem Altern M* 2018; 18(1):7.
112. Panahi Y, Khalili N, Sahebi E, et al. Curcuminoids plus piperine modulate adipokines in type 2 diabetes mellitus. *Curr Clin Pharmacol* 2017;12: 253-8.
113. Kantarjian HM, Roboz GJ, Kropf PL, et al. Guadecitabine (SGI-110) in treatment-naive patients with acute myeloid leukaemia: phase 2 results from a multicentre, randomised, phase 1/2 trial. *Lancet Oncol* 2017;18:1317-26.
114. Molokie R, Lavelle D, Gowhari M, et al. Oral tetrahydrouridine and decitabine for non-cytotoxic epigenetic gene regulation in sickle cell disease: a randomized phase 1 study. *PLoS Med* 2017;14:e1002382.
115. Watson CJ, Horgan S, Neary R, et al. Epigenetic therapy for the treatment of hypertension-induced cardiac hypertrophy and fibrosis. *J Cardiovasc Pharmacol Ther* 2016;21:127-37.
116. Swayze EE, Siwkowski AM, Wancewicz EV, et al. Antisense oligonucleotides containing locked nucleic acid improve potency but cause significant hepatotoxicity in animals. *Nucleic Acids Res* 2007; 35:687-700.
117. Nguyen AT, Taranova O, He J, Zhang Y. DOT1L, the H3K79 methyltransferase, is required for MLL-AF9-mediated leukemogenesis. *Blood* 2011;117:6912-22.

**KEY WORDS** cardiac hypertrophy, epigenetics, heart failure

