Published in final edited form as:

Epigenomics. 2015 April ; 7(2): 321–330. doi:10.2217/epi.14.60.

Histone methylations in heart development, congenital and adult heart diseases

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Abstract

Heart development comprises myocyte specification, differentiation and cardiac morphogenesis. These processes are regulated by a group of core cardiac transcription factors in a coordinated temporal and spatial manner. Histone methylation is an emerging epigenetic mechanism for regulating gene transcription. Interplay among cardiac transcription factors and histone lysine modifiers plays important role in heart development. Aberrant expression and mutation of the histone lysine modifiers during development and in adult life can cause either embryonic lethality or congenital heart diseases, and influences the response of adult hearts to pathological stresses. In this review, we describe current body of literature on the role of several common histone methylations and their modifying enzymes in heart development, congenital and adult heart diseases.

Keywords

cardiac hypertrophy; congenital heart disease; demethylase; epigenetics; heart development; histone; methyltransferase

Epigenetics in modern term refers to study of molecular factors and processes that regulate genomic activities independent of DNA sequence. Well-studied epigenetic mechanisms comprise histone post-translational modifications (PTMs), ATP-dependent chromatin remodeling, DNA methylation and microRNA. As this review focuses on the role of histone methylation involved in cardiac development and congenital and adult heart diseases, only aspects of histone methylation/demethylation and their modifying enzymes will be described here. The subject of other epigenetic mechanisms can be found in several excellent articles [1–6].

Methylation of histone was originally identified in the 1960s. The methylation enzyme was not known until the first lysine methyltransferase (KMT) family Su(var)3–9 was discovered [7]. Histone methylation was thought to be a permanent PTM until the discovery of first histone lysine demethylase (KDM), LSD1/KDM1A [8,9]. Histone can be methylated at both

lysine (K) and arginine residues. Well-studied histone methylation residues include K4, K9, K27, K36 and K79 at histone H3, and K20 of histone H4. Lysine residues can be methylated to different degrees to include mono(me1)-, di(me2)- or trimethyl(me3) groups. The sequential addition of methyl moieties can occur, but it is not essential requirement since some KMTs have the capacities to mono-, di- or tri-methylate unmodified lysine residues. Histone methylation/demethylation can result in either gene activation or gene repression depending on methylated lysine residues and the degree of methylation. Since histone methylation occurs in the context of other surrounding PTMs, the ultimate functional output of a specific histone lysine methylation event will depend on the collective abundance, regulation and context of all surrounding PTMs (the so-called histone code) [10].

So far, there are 33 KMTs and 21 KDMs identified in humans [11]. KMT has the SET-domain-containing enzymes (KMT1-3, KMT5-8) and the Dot1L/KMT4 family. KDM has the flavin adenine dinucleotide-dependent amine oxidases (KDM1/LSD) and Jumonji C (JmjC) domain-containing demethylases (KDM2–8) family. KMTs and KDMs have a high degree of specificity for particular lysine residues and the degree of methylation. However, the specificity of some KMT and KDMs can be context dependent and affected by its interacting partners. For example, LSD1 has dual substrate specificity (H3K4me2/1 and H3K9me2/1). LSD1 demethylates H3K4me1/2 and generally represses transcription when it is a part of transcriptional co-repressor complexes that contain CoREST and/or HDAC1/2 [12]. When bound to androgen receptor in prostate cancer cells, LSD1 collaborates with JmjC demethylases (JMJDs) to remove the repressive H3K9me2/1 marks and activates androgen receptor-dependent gene transcription [13]. Different histone methylation patterns can provide specialized binding surfaces that recruit protein complexes containing chromatin remodeling and transcriptional activation/repression activity. While lysine methylation at H3K9, H3K27 and H4K20 is, in general, associated with regions of transcriptionally silenced heterochromatin, methylation at H3K4, H3K36 and H3K79 is normally associated with transcriptionally active euchromatin [14].

Heart development, congenital heart disease & cardiac hypertrophy

The development of the vertebrate heart is a complex process that requires concurrent differentiation of multiple cell types including endothelial cells, smooth muscle cells and cardiomyocytes. The process of heart formation may be considered an additive one, in which additional layers of complexity are added in the form of modular elements with distinct genetic and epigenetic control [15–18]. Five major stages of heart development in mice have been identified including cardiac crescent stage at E7.75, linear heart tube formation at E8.0, chamber initiation and formation from E9.5 and chamber maturation and septation from E12.5 and valve formation from E12 to birth. Two populations of cardiac progenitor cells can be identified at the cardiac crescent stage, first heart field (FHF) and a more medial region called second heart field (SHF) that lie adjacent to each other [16]. The two heart fields are spatially and molecularly distinct. FHF gives rise to the initial linear heart tube that is destined to become the myocardium of the left ventricle (LV). Progenitor cells in the SHF remain undifferentiated until they are added later to the heart tube to give rise to the right ventricle (RV) and the outflow tract (OFT). Cardiac neural crest cells control SHF development and form endocardial cushions in the OFT. The heart development is

controlled by a group of core cardiac transcription factors (Mesp1, Isl1, Nkx2.5, Mef2c, Tbx1, Gata4, Foxa2/c1/c2/h1 and Hand2) and growth factors (Fgf8/10, Wnt3a/5a/11, Bmp2/4/7, Shh and others) in a temporal and spatial-dependent manner [15–18]. Mesp1 sits on the top of the cardiac transcription factor hierarchy and represents the earliest marker of cardiovascular progenitors including derivatives of the first and second heart fields [19]. Mesp1 and Mesp2 are required for establishing embryonic mesoderm that gives rise to heart, somites and gut. Mesp1 cells are converted further into cardiac progenitors by Isl1, Nkx2.5, Gata4, Mef2C and Tbx5. Mutation and abnormal expression of these factors during heart development have been shown to lead to inherited forms of congenital heart diseases (CHD) such as septal defects, atrioventricular malformations or developmental absence of right or left heart [18,20,21].

Adult cardiomyocytes are considered terminally differentiated with limited proliferative capacity. In response to physiological stimuli such as exercise and pregnancy, the heart undergoes adaptive and reversible hypertrophy via increase in cardiomyocyte size in order to pump sufficient blood to meet increased hemodynamic requirement [22]. The adaptive cardiac hypertrophy becomes maladaptive and irreversible under pathological conditions including chronic hypertension, myocardial infarction, obesity, diabetes, aging and genetic mutations. Pathological cardiac hypertrophy loses its plasticity with diminished ability to mount adaptive response to meet systemic demands and is a primary risk factor for heart failure and an adverse prognosis. At molecular level, pathological cardiac hypertrophy is marked by dramatic alteration of gene expressions associated with contractile apparatus and metabolism [23]. Fetal genes such as β MHC that are normally expressed in embryonic and fetal heart and silenced in adult cardiomyocytes are reactivated whereas many genes expressed in differentiated cardiomyocyte including adult isoforms α MHC are repressed. Alteration of gene expression may play a causative role in pathological cardiac hypertrophy and heart failure [22].

Emerging evidence indicates that lysine methylation balance is critical in maintaining genome integrity, gene regulation and cancer evasion [11]. Genome-wide association studies, genomic sequencing and mapping identified mutations, copy number variation and aberrant gene regulation of KMTs and KDMs that are associated with embryonic development, aging, cancer and neurological disorders [11,24]. Histone methylation has also shown to play important roles in cardiac lineage specification and differentiation [25], heart development [26,27] and pathogenesis of congenital and adult heart diseases [26–29]. In an *in vitro* step-wise cardiomyocyte differentiation study in which mouse ES cells were induced to become mesodermal cells, then cardiac precursors and finally differentiated cardiomyocytes, Warmstad *et al.* found that while multiple dynamic chromatin patterns were observed during the differentiation process, a specific chromatin pattern was found for most mesodermal-specific genes [25]. Genes with metabolic functions also found to share a similar chromatin pattern, suggesting that epigenetic regulation may play an important role to ensure that functional gene modules are synchronized to produce robust and coordinated expression. Furthermore, H3K4me1 was found to mark the transcriptional start sites (TSS) of a group of genes at early differentiation stages that are committed to cardiac lineage and activated later via acquisition of H3K4me3 [27]. Alteration of the levels of histone

H3K4me3 and H3K9me3 is also found to correlate with heart failure [30]. In the following sections, we will review five most characterized histone methylations in the literature and the evidences that suggest dysregulation of these lysine methylations may play a causative role in promoting CHD and adult cardiac hypertrophy (Table 1).

H3K4 methylation

H3K4 methylation is generally associated with transcriptional active genes. Trimethylated H3K4 (H3K4me3) at the TSS of an active gene correlates strongly with the histone acetylation, transcription rate and active RNA polymerase II binding. H3K4me3 at TSS may play a functional role in transcription initiation as H3K4me3 can bind the PhD domain of TAF3, facilitating the recruitment of RNA polymerase II [31]. H3K4me3 is also found together with H3K27me3 at promoters and enhancers of poised key developmental genes in pluripotent ES cells [32,33]. In vertebrates, the majority of H3K4me2/me1 co-localize with H3K4me3 at the TSS. H3K4me1/2 can also be found in distal enhancer region. Although the functional consequence of H3K4me2/1 at TSS and enhancer regions remains elusive, it may mark lineage-specific genes at early differentiation stages such that they are poised to be activated via acquisition of H3K4me3 at later differentiation stages [25].

H3K4 methylation is maintained by methyltransferases (KMT2A-H, KMT3E-G, KMT7 and KMT8B) and demethylase (KDM1A-B, KDM5A-D) [11,34]. Mammalian H3K4 methyltransferases can be classified into two groups: MLL family proteins containing SET domain related to the SET domain of yeast Set1 and *Drosophila* Trx (Set1A/B, MLL1–4), and other H3K4 methyltransferases – those unrelated yet able to methylate H3K4 (ASH1, SMYD, SET7/9 and PRDM9). MLL proteins are in COMPASS-like complexes capable of mono-, di- and tri-methylating H3K4 [35]. The COMPASS-like complex contains three additional core subunits (WDR5, RbPB5 and ASH2). While the four-component minimal complex is sufficient to catalyze H3K4 methylation, several other proteins are found in biochemically purified COMPASS-like complexes including MEN1, UTX, PTIP, NCOA6, PA1, Dpy30 and/or HCF1. The precise biological function of the noncatalytic subunits co-purified with COMPASS-like complexes remains elusive. Their possible functions include context-dependent recruitment of the complex to target genes, and substrate recognition and presentation. Many of the noncatalytic subunits in the complex are H3K4 methyl readers that are coupled to other chromatin modification mechanisms necessary to execute specific biological functions [35].

De novo mutations (present in the probands but absent in both parents) of many genes that produce and read methylated H3K4 were identified recently in patients with CHD [36]. These genes include *MLL2/KMT2D*, *CHD7*, *WDR5*, *RNF20*, *UBE2B* and *USP44*. *CHD7* and *WDR5* are H3K4 methylation readers that bind H3K4me1/me2. *RNF20* and *UBE2B* are subunits of H2BK120 ubiquitination complex. *USP44* is H2BK120 deubiquitinase. Ubiquitination at H2BK120 is required for H3K4 methylation [35]. *MLL2* mutations were previously found in majority of patients with Kubaki syndrome that includes atrial and ventricular septal defects and aortic coarctation [37,38]. *Mll2* plays an important role during early embryogenesis as mice are embryonic lethal prior to E11.5 with wide-spread apoptosis when *Mll2* is deleted germline and are normal when deleted after E11.5 [39,40]. Mice with

mutant *Mll2*^{M2628K} were generated through N-ethyl-N-nitrosourea mutagenesis screens [41]. *Mll2*^{M2628K/M2628K} mice are embryonic lethal between E8.5 and E11.5 with abnormal heart looping, pericardial effusion, exencephaly and various head abnormalities and anterior truncation defects. *Mll2*^{M2628K/+} mice are viable and have diabetes in adulthood [41]. *CHD7* mutation is found in a majority of children clinically diagnosed with CHARGE syndrome that is characterized by coloboma of the retina, heart defects, atresia of the choanae, retarded growth and development genital and ear abnormalities [42]. Most cardiovascular anomalies of CHARGE syndrome are related to pharyngeal arch artery (PAA) defects or cardiac outflow tract malformations. Mechanistic studies that link directly the association of these mutations with H3K4 methylation to cardiac gene expression are lacking at present. However, it was suggested that CHD7 may regulate *Tbx1*-targeted genes. Some of the cardiovascular defects of CHARGE syndrome are recapitulated in *Chd7*^{+/-} mice [43]. Both *Tbx1* and *Chd7* heterozygous mice had hypoplasia of PAA. *Chd7*^{+/-}; *Tbx1*^{+/-} compound heterozygote showed greater defect in PAA than either *Chd7* or *Tbx1* heterozygote alone, suggesting that *Chd7* and *Tbx1* may interact genetically. PTIP is a subunit in MLL3/4(KMT2C/D) complexes. PTIP promotes assembly of the MLL3/4 complexes and H3K4 methylation at a PAX2 DNA binding site via binding to PAX2 transcription factor [44]. Deletion of *Ptip* in mice resulted in developmental arrest at gastrulation stage [44] with reduced levels of H3K4me3 in the whole embryo [43] and embryonic stem cells [45]. Conditional deletion of *Ptip* in differentiated cardiomyocyte in adult mouse heart resulted in reduced expression of *Kcnip2* and H3K4me3 occupancy at the *Kcnip2* promoter. *Ptip* mutant hearts had conduction defect and greater susceptibility to calcium-based ventricular arrhythmias [29].

SMYD family proteins (SMYD1–5/KMT3) contain SET domains required for histone methyltransferases activity and MYND domains that mediate interactions with proteins involved in transcriptional regulation and signal transduction. Smyd2 has been shown to monomethylate H3K4 and H3K36 [46,47]. Smyd3 di- and tri-methylates H3K4 [48]. Smyd2 is also shown to methylate nonhistone proteins including p53, Rb and Hsp90. The substrate specificity for Smyd1, 4 and 5 proteins remains elusive. Smyd1/Bop is mostly expressed in skeletal and cardiac muscles and is a striated muscle-specific regulator of myogenic differentiation and myosin assembly [49]. Deletion of *Smyd1* in mice disrupts cardiac differentiation and morphogenesis, resulting in embryonic lethality. *Smyd1*-null embryonic hearts have a hypoplastic RV with reduced expression of *Hand1* and *Irx4*, two genes required for myocardial growth and differentiation [49]. Smyd1 was shown to interact with transcription factor skNAC to regulate downstream gene expression. *skNAC* knockout mice have similar embryonic phenotype to that of *Smyd1*-null mice [50]. Smyd1 also binds myosin and is localized at the M-band region in the center of the sarcomere. Mutation in *Smyd1* in zebrafish causes disturbed sarcomere assembly in the heart and fast-twitch skeletal muscle [51]. Smyd2 methylates Hsp90, promoting interaction of a Smyd2-methyl-Hsp90 complex with the N2A-domain of titin [52]. Although no cardiac phenotypes are observed in *Smyd2*-null mice at baseline [53], it may be required for protection of the sarcomeric I-band region and myocyte organization under conditions of mechanical or biochemical stress [52]. A role for Smyd3 in the development of cardiac and skeletal muscle was shown in zebrafish [54].

H3K4 methylation can be reversed by members of JARID1/KDM5 (from K4me3/2 to K4me1) or LSD/KDM1 (from K4me2/1 to K4me0) family of histone demethylases [11]. H3K4 demethylases can be recruited to specific genomic loci through their intrinsic reader domains or gene-specific transcriptional factors. Polymorphic variants in the *LSD1* gene are associated with salt-sensitive hypertension in human. Haploid-deficient *Lsd1* mice had higher blood pressure under high-salt diet than control wild-type mice. Levels of eNOS and guanylate cyclase expression are reduced in the *Lsd1*^{+/-} mouse aorta and heart [55]. *De novo* mutations in *KDM5A/5B* and *KDM6A* were identified in human patients with CHD [36], although mechanisms linking mutations of these demethylases to histone H3K4 methylation and to target gene expression remain unknown.

H3K27 methylation

H3K27 methylation is usually associated with gene repression and appears mostly in Polycomb-associated repressive chromatin. Polycomb group proteins (PcG) are a family of proteins first discovered in fruit flies that silence gene expression. In mammals, two PcG complexes are characterized: Polycomb repressive complex 1 (PRC1) and PRC2. H3K27me3 is catalyzed by the catalytic subunit EZH2 in PRC2 [56]. H3K27me3 can in turn recruit PRC1 that monoubiquitinates H2A at K119 (H2AK119ub1). H2AK119ub1 functions to inhibit RNA polymerase II elongation [57]. H3K27me3 is demethylated by KDM6A/UTX.

H3K27me3 represses many developmental genes in self-renewing ES cells and in differentiated adult organs [32,33]. In ES cells, H3K27me3 can form ‘bivalent’ marks with H3K4me3 on promoters and enhancers of key developmental genes that are poised to undergo transcriptional activation/deactivation upon developmental cues and/or environmental stimuli. When the silencing H3K27me3 mark is removed and the activating H3K4me3 is retained, ‘poised’ genes are activated and ES cells are committed to a particular lineage. Conversely, bivalent domains of other genes required for the commitment of ES cells to different lineages remain silenced with H3K27me3 retained along with other repressive marks including H3K9me3, and/or 5mC DNA methylation. The function of bivalent marks and their associated protein complexes are suggested to help maintaining bivalent loci in a state that is both responsive to developmental cues and at the same time refractory to subthreshold noise [32].

During heart development, cardiac progenitor cell commitment and subsequent growth and differentiation require accurate activation of a gene program in a precise temporal and spatial manner. H3K27me3 marks promoters of cardiac-specific genes in ES cells [25,33,58]. It is necessary to remove H3K27me3 in order for ES cells to differentiate to a cardiac lineage as ES cells lacking H3K27 demethylase Utx failed to express cardiac-specific genes under cardiac differentiation conditions *in vitro* [58]. *Utx*-null mice are embryonic lethal with failed cardiac looping morphogenesis and chamber development at E9 [58]. Utx was shown to interact with and function as a co-activator of core cardiac transcription factors including SRF, Tbx5, Nkx2.5 and GATA4. Furthermore, Utx interacts with ATPase Brg1 and facilitates the recruitment of Brg1-containing Swi/Snf chromatin remodeling complex to the cardiac genes. Swi/Snf complex was previously shown to play an

important role in heart development [59]. Partial or complete deletion of *UTX* is also implicated in human CHD [36].

While removal of H3K27me3 is required for activation of cardiac-specific genes during cardiac cell differentiation, maintaining H3K27 methylation at noncardiac genes to repress their expression is also essential for proper heart development. Two groups have recently shown that inactivation of H3K27 methyltransferase *Ezh2* in differentiating cardiomyocytes in mice causes CHD [26,27]. When *Ezh2* was deleted in cardiac progenitor cells in the SHF using *Mef2cAHF*-cre at E7.5, mice developed hypertrophied and fibrotic hearts after birth [27]. Deletion of *Ezh2* in differentiating ventricular cardiomyocytes using *Nkx2.5*-cre resulted in perinatal lethality. Mutant mouse hearts had persistent hypertrabeculation, right ventricular hypoplasia, atrial and ventricular septal defects and myocardial fibrosis [26]. In both studies, noncardiac gene transcriptions including those of *Ink4a*, *Six1*, *Isl1* and *Pax6* were upregulated. H3K27me3 occupancies at the promoters of these genes were downregulated. These studies underscore the dynamic roles of H3K27 methylation in heart development and pathogenesis of CHD.

H3K9 methylation

H3K9 methylation is the second most conserved feature of silenced chromatin in eukaryotes after histone acetylation [60]. Unlike H3K27 methylation that appears mostly in Polycomb-associated repressive chromatin in gene-rich region, H3K9 methylation is enriched in transcriptionally inert heterochromatin that includes satellite repeats in telomeres and pericentromeres. Trimethylated H3K9 (H3K9me3) can bind to heterochromatin protein-1 (HP1) that further recruits other proteins to assemble chromatin into compact or 'closed' heterochromatic structures, leading to silenced gene expression [61,62]. H3K9 methylation is also found to participate in transcriptional repression of genes in euchromatic regions through epigenetic modification of promoters [28] and in the repression of illegitimate initiations of transcription inside of actively transcribed loci [60]. H3K9me3 can co-exist with other repressive histone methylation, including H3K27me3 and H4K20me3 on silenced chromatin [63,64]. These marks are often found on macrodomains that have characteristics of a facultative heterochromatin and can lose its condensed structure and become transcriptional permissive in response to specific developmental and environmental cues.

Methylation of H3K9 is maintained by KMT1, KMT2H, KMT8A/D, KDM3, KDM4 and KDM7. Imbalance of H3K9 methylation due to mis-regulation of either KMTs or KDMs is associated with numerous malignancies including B-cell lymphoma, medullo-blastoma, AML, breast, lung and prostate cancers and neurological disorders such as mental retardation, epileptic seizures and autism. H3K9 KMT and KDM are involved in cardiac diseases and development as well. H3K9me3-specific demethylase JMJD2A/KDM4A is upregulated in human patients with hypertrophic cardiomyopathy [28]. Majority of the cardiomyocyte undergo terminal differentiation after birth with limited proliferative capacity. Many fetal gene expressions are repressed in normal adult hearts and re-expressed again in response to hypertrophic stress signals. Since H3K9me3 is associated with gene silencing, its level may be downregulated in hypertrophic heart as the fetal gene programs

are re-activated. This hypothesis was tested in mouse model of cardiac hypertrophy where the transverse aorta is constricted (TAC) to induce pressure overload in the left ventricle [28]. The global H3K9me3 level was downregulated in hypertrophic mouse heart after 3 weeks of TAC (authors' unpublished results). Jmjd2a promoted TAC-induced cardiac hypertrophy when over-expressed in postnatal cardiomyocytes and blunted hypertrophic response when deleted from the heart [28]. Mechanistically, Jmjd2a was shown to bind promoters of fetal genes (*Anp* and *Bnp*) and *Fhl1* in response to hypertrophic stimuli and activated their transcription through binding to transcription factors SRF/myocardin. Fhl1 is a key component of the mechanotransducer machinery in the heart. *Fhl1* KO mouse has blunted hypertrophic response to TAC-induced injury [65]. The demethylase activity of Jmjd2a is required to promote transcription of fetal genes and *Fhl1* [28]. Activation of *Fhl1* in TAC-induced hypertrophic heart by Jmjd2a is associated with downregulation of H3K9me3 at *Fhl1* promoter. It is interesting to note that although the global H3K9me3 was altered in response to hypertrophic stress signal, there were only a limited number of genes whose transcriptions were changed due to *Jmjd2a* deletion [28]. It is possible that other Jmjd2 proteins are involved in H3K9me3 regulation and Jmjd2a only regulates a specific subset of genes. Alternatively, Jmjd2a may regulate global H3K9me3 methylation but only a subset of the targets has altered gene transcription due to the effect of other PTMs. A genome-wide profiling of Jmjd2a-binding sites in the heart during hypertrophic remodeling is needed to test these hypotheses.

Jmjd2a may play an important role in embryonic heart development as well. Knockdown of *Jmjd2A* in chick embryos by *Jmjd2A*-specific antisense morpholino oligonucleotides was shown to dramatically down-regulate the expression of neural crest specifier genes including *Sox10*, leading to defects in neural crest derivatives at later stages of embryonic development [66]. We observed no embryonic defects in global deletion of *Jmjd2a* in mice of mixed genetic background (C57/Bl6:129). However, a partial embryonic lethality was observed in pure C57/Bl6 mouse. Some of the *Jmjd2a*-null embryos at E10.5 had abnormal hearts with various cardiac defects including thin myocardium and underdeveloped RV (authors' unpublished results). Since development of RV is derived from SHF that is controlled by cardiac neural crest cells, the embryonic phenotype of *Jmjd2a*-null mouse may be due to the defects in neural crest cells, a hypothesis needed to be tested in the future. The wide spectrum of embryonic phenotypes of *Jmjd2a*-null embryos and dependency of the genetic background may implicate genomic context, maternal-fetal environment and other factors that were proposed to explain why an identical gene mutation give rise a variety of distinct malformations [20]. Incomplete penetrance may be caused by variability of gene expression [67], as in some embryos, alteration of *Jmjd2a*-targeted gene expressions may not be sufficient to reach a threshold that causes a phenotypic change.

H3K36 methylation

H3K36me3 is normally found in gene bodies of actively transcribed genes. However, H3K36 methylation has also been implicated in transcriptional repression, alternative splicing and many other biological processes [68,69]. Whether H3K36me3 acts as a transcriptional activating or inhibitory signal may depend on the context of additional PTM marks and their corresponding reader proteins [69]. H3K36 can be methylated by WHSC1.

Deletion of a critical region of human chromosome 4q16.3 containing WSHC1 gene is associated with Wolf-Hirschhorn syndrome that is characterized by craniofacial malformations, growth retardation, learning disability and congenital heart defects. *Whsc1*^{-/-} mice are perinatal lethal with marked growth retardation and die within 10 days after birth. *Whsc1*-null mice display a variety of atrial and ventricular septal defects that manifest those in Wolf-Hirschhorn patients. *Whsc1* can interact with transcription factors such as Sal4 and Nanog in ES cells. In embryonic heart, *Whsc1* was found to interact with Nkx2.5 to repress *Pdgfra* expression. Loss of *Whsc1* resulted in reduction of H3K36me3 at the *Pdgfra* locus and upregulation of *Pdgfra* [70].

H3K79 methylation

The function of H3K79 methylation is inferred from the studies of its methyltransferase Dot1 and its mammalian homolog DOT1L/KMT4 since knock out of *Dot1* in yeast, flies and mice resulted in complete loss of H3K79 methylation [71]. Dot1/DOT1L-mediated H3K79 methylation has been linked to telomeric silencing in yeast and active transcription. Cardiac-specific knockout of *Dot1l* caused dilated cardiomyopathy (DCM) with chamber dilation and systolic dysfunction [71]. Mechanistically, Dot1l was shown to activate the transcription of dystrophin and Dot1l-mediated H3K79 methylation is required for dystrophin expression. It was also shown that dystrophin is the major downstream target of *Dot1l* since ectopic expression of a functional truncated form of dystrophin can rescue the DCM phenotype observed in *Dot1l*-cardiac-specific knockout mice. DOT1L may also play an important role in human DCM as *DOT1L* is significantly lower in human idiopathic DCM heart tissues compared with normal ones [71]. Unlike other histone lysine methylation, H3K79-specific lysine demethylase is yet to be discovered.

Conclusion & future perspective

Emerging evidence indicates that aberrant histone methylations due to dysregulation of their modifying enzymes (methyltransferase and demethylases) and defective methyl readers can lead to congenital and adult heart diseases. With the advancement of modern genomic technologies, more and more mutations of epigenetic regulators are being identified. Mechanistic studies linking association of mutation with lysine methylation to downstream gene expression using transgenic animal models will be needed in order to understand which and how alterations in epigenetics cause, rather than correlate with, congenital and adult heart diseases. Unlike most of the cell types that were profiled for histone modifications, adult cardiomyocytes are lineage determined and ‘terminally’ differentiated with little cell-cycle activities; the epigenetic regulatory mechanisms are most likely to differ from those identified so far in pluripotent ES cells or lineage determined cycling cells. So far, genome-wide change of histone methylations in differentiated adult cardiomyocytes in response to hypertrophic stimuli is lacking and needs to be explored in order to discover key epigenetic events underlying adult cardiac hypertrophy and heart failure. Studies in humans and animals indicate that early exposure of nutrients at the time of conception and during pregnancy can lead to lasting epigenetic changes in their off-springs with increased risk of disease in adult life [72–74]. The activities of many epigenetic enzymes require co-factors and are hence potentially susceptible to changes in nutrient intake and metabolism [75]. It

will be worthy to explore in the future how enzymatic activity of epigenetic regulators senses the cellular metabolism and alters the epigenetic mark in response.

Acknowledgments

Financial & competing interests disclosure

Work in Z-P Liu's laboratory is supported by grant from NIHRO1 (HL109471) and Cancer Prevention and Research Institute of Texas (CPRIT RP120717). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

Heart development, congenital heart disease and cardiac hypertrophy

- Heart development is regulated by core cardiac transcriptional factors and epigenetic mechanisms including histone lysine methylation. Mutation of transcription factors and methyltransferases/demethylases can cause embryonic lethality, congenital and adult heart diseases.

H3K4 methylation

- H3K4 methylation is generally associated with transcriptional active genes. Mutations in H3K4 methyltransferases and demethylases are found to be associated with several congenital heart diseases including Kubaki syndrome and CHARGE syndrome.

H3K27 methylation

- H3K27 methylation is usually associated with Polycomb-associated repressive chromatin. In ES cells, H3K27me3 can form 'bivalent' marks with H3K4me3 on promoters and enhancers of key developmental genes that are poised to undergo transcriptional activation/deactivation upon developmental cues and/or environmental stimuli. Mutation of H3K27me3 demethylase UTX in mice resulted in embryonic lethality due to defective heart morphogenesis. Mutation of H3K27 methyltransferase Ezh2 can lead to congenital and adult heart diseases.

H3K9 methylation

- H3K9 methylation is usually associated with transcriptionally inert heterochromatin. It also participates in transcriptional repression of genes in euchromatic regions. H3K9me3 demethylase JMJD2A promotes cardiac hypertrophy in response to pressure-overload due to transaortic constriction.

H3K36 methylation

- H3K36me3 is normally found in gene bodies of actively transcribed genes. H3K36 methylation has also been implicated in transcriptional repression, alternative splicing and many other biological processes. Lack of H3K36 methyltransferase WSHC1 may lead to Wolf-Hirschhorn syndrome.

H3K79 methylation

- Loss of H3K79 methylation due to inactivation of *Dot1l* in the mouse heart caused dilated cardiomyopathy (DCM) with chamber dilation and systolic dysfunction.

Table 1

Selected histone methylations and their modifying enzymes involved in heart development, congenital heart diseases and cardiac hypertrophy.

Methylation	Modifier	Function and mechanism of the modifier	Cardiac phenotype	Ref.
H3K4	MLL2	Methylates H3K4me3 at 'bivalent' loci and also active gene promoters	Mutation of <i>MLL2</i> may cause Kabuki syndrome in patients that have atrial and ventricular septal defects, and aortic coarctation	[37,38]
			<i>Mll2</i> -null mice are embryonic lethal	[39–40]
			Adult <i>Mll2</i> ^{M2628K/+} mice have Type 2 diabetes	[41]
	SMYD1	Contains SET domain, interacts with skNAC and regulates Hand2 and Irx4	<i>Smyd1</i> -null mice have hypoplastic right ventricle, knockdown in zebra fish resulted in disrupted myofibril formation	[47,48]
	PTIP	Part of MLL3/4 complexes, may recruit MLL3/4 complexes to target genes such as Kcnip2	Deletion of <i>Ptip</i> in adult cardiomyocyte resulted in defective conduction system and greater susceptibility to Ca ²⁺ -based ventricular arrhythmia	[43]
	LSD1	Demethylates H3K4me1/2	<i>Lsd1</i> ^{+/-} mice have salt-sensitive hypertension	[53]
H3K27	Ezh2	Establishes H3K27me3 at noncardiac loci to repress their expression during cardiomyocyte differentiation	Deletion of <i>Ezh2</i> in cardiac progenitor cells leads to thinned myocardial walls and ventricular septation defects	[26]
			Deletion of <i>Ezh2</i> in second heart field, a more restricted pool of cardiac progenitor cells, resulted in enlarged heart after birth	[27]
	UTX	Removes H3K27me3 at cardiac-specific enhancers to activate cardiac gene expression during heart development	<i>Utx</i> -null mice are embryonic lethal at E9.0 with defective loop and chamber morphogenesis	[58]
H3K9	Jmjd2a	Removes H3K9me3 at <i>Fhl1</i> and other cardiac fetal gene loci	Deletion of <i>Jmjd2a</i> in adult cardiomyocyte blunts pressure overload induced cardiac hypertrophy	[28]
H3K36	WHSC1	Establishes K3K36me3 at <i>Pdgfra</i> locus and represses <i>Pdgfra</i> expression together with Nkx2.5	<i>Whsc1</i> -null mice are perinatal lethal with a variety of atrial and ventricular septal defects	[69,70]
H3K79	Dot1L	Methylates H3K79, regulates telomere elongation and cell proliferation	<i>Dot1L</i> -null mice have cardiac dilatation, yolk sac angiogenesis defects, embryonic lethal at E9.5–E10.5	[71]