Abstract. Histone post-translational modifications (PTM) as one of the key epigenetic regulatory mechanisms that plays critical role in various biological processes, including regulating chromatin structure dynamics and gene expression. Histone lysine methyltransferase contributes to the establishment and maintenance of differential histone methylation status, which can recognize histone methylated sites and build an association between these modifications and their downstream processes. Recently, it was found that abnormalities in the histone lysine methylation level or pattern may lead to the occurrence of many types of cardiovascular diseases, such as congenital heart disease (CHD). In order to provide new theoretical basis and targets for the treatment of CHD from the view of developmental biology and genetics, this review discusses and elaborates on the association between histone lysine methylation modifications and CHD.

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1. Introduction

Congenital heart disease (CHD) involves the anatomical structure abnormality caused by the formation of obstacles or the abnormal development of the heart and great vessels during the period of embryonic development, or a group of congenital malformations with actual or potential influence on heart function arising from the open tunnels which should have self-closed after childbirth. CHD mostly occurs during 2-8 weeks after impregnation, and it is the most common cardiovascular malformation affecting children; it severely affects the health of infants and young children (1-4). At present, CHD is regarded as a multigene disease influenced by the environment and heredity; however, the pathogenesis of the disease and the underlying molecular mechanisms and interactions between genes remain unclear (5-8). Heart development is a very complex process, involving the expression of numerous genes at specific time points in the process of embryonic development, and it is regulated by many transcription factors (9,10). The realization of this process is not only determined by gene sequences, but is also largely generated by the transformation of epigenetics. In addition, an increasing number of studies have found that children with CHD have an extremely low occurrence rate of gene mutation, which can only explain a small number of CHD cases, as there is no pathogenic gene transformation for the majority of CHD cases (11-13). Some recent studies have found that ‘epigenetics’ may very likely participate or play an important role in the occurrence of CHD (14-16).

Epigenetics suggests that DNA sequence undergoes no transformation, but the gene expression occurs by heritable transformation, which is the other heritable material transformations in the cells apart from the heritage information and with stable heredity in the process of cell development and proliferation (17-19). Epigenetics is mainly the reversible and heritable transformation of gene function with the DNA sequences of the nucleus unchanged, and these transformations include DNA...
Arg methylation is a comparatively dynamical mark, and is
(H4K20), Arg locus 2, 17, 26 of histone H3 (H3R2, H3R17 and
H3K9, H3K27, H3K36 and H3K79), Lys locus 20 of histone H4
Lys and Arg. Lys locus 4, 9, 27, 36 and 79 of histone H3 (H3K4,
histone modifications and regulator gene expressions (29,58).
Lys methylation has the gene expression regulatory function similar to the
DNA genetic code, and plays an important role in the process of
growth and development (32-34).

In recent years, studies have shown that histone lysine
methylation is not only closely related to tumor occurrence and
development, developmental defects, senile dementia, cardiac
hypertrophy and other clinical diseases, but also participates in
the occurrence of CHD, influencing the development of heart
structure and CHD candidate gene expression (35-45). For
this reason, this review aims to summarize the new progress
of CHD epigenetic mechanism research from the aspect of
histone lysine methylation modification, in order to provide a
new scientific basis for the prevention and treatment of CHD.

2. Histone methylation

Histone methylation involves the methylation occurring
at histone H3 and the N-terminal of H4 arginine (Arg) or
lysine (Lys) residues, catalyzed by histone methyltransferase.
The function of histone methylation is mainly reflected in
heterochromatin forming, genomic imprinting, X chromosome inactivation and transcriptional control (46-52). Apart
from histone methyltransferase, the histone demethylase is
also found (53,54). At first, it was considered that the histone
methylation effect was stable and irreversible; however, the
existence of methyltransferase renders the process of histone
methylation more dynamical.

Modification sites of histone methylation. Histone methylation
can occur on of Lys and Arg histone residues, catalyzed by
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methylation effect was stable and irreversible; however, the
existence of methyltransferase renders the process of histone
methylation more dynamical.

Related enzymes of histone methylation. Arginine methylation
occurs at the equal locus of histone H3R2/R17/R26 and H4R3,
and plays a promoting effect on gene expression (60). When
the methylation process occurs, histone arginine methyltransferase
or protein arginine methyltransferase (PRMT), as the collabora-
tive activity factor, is recruited into the promoter region of
the target gene and thus activates gene expression (73-75). The
PRMT of catalyzing histone arginine methyltransferase includes
two categories: the first type of PRMT being catalyzed to form
single methylation arginine and unsymmetrical double meth-
ylation arginine, and the second type of PRMT being catalyzed
to form single methylation arginine and symmetrical double
methylation arginine (76,77). The family of PRMT includes
PRMT1, PRMT3, RMT4/HMT, PRMT4/CAMRl and PRMT5.
K4, K9, K27, K36, K79 of histone H3 and K20 of H4 can be
methylated. The methylation level is regulated and executed
by a type of methyltransferase with SET structural domain with
highly conserved nucleus and the pre-SET and post-SET
structural domain with abundant cysteine sequence (31,78,79).
The demethylation process occurs with the aid of SET domain-containing histone demethylase (JHDM) (81,82).

3. Histone lysine methylation and heart development

Epigenetic modification, including methylation and acetylation,
plays an important role in the regulation of gene expression. Studies have indicated that histone methylation can reduce or
increase its affinity for charged DNA, loosen or tighten the
chromatin structure to affect the accessibility and interactions
between transcriptional factors and DNA templates, ultimately
promote or inhibit gene expression (83,84). Recent evidence
suggests that histone methylation in normal and aberrant heart development (85-87). A recent study demonstrated that changes in histone methylation levels in histone H3 that
binds with critical promoter parts of the ssTnI gene can cause
the corresponding changes in ssTnI gene expression, which
indicated that histone methylation was involved in the
regulation of myofibril gene expression in the heart during
development (88). Additionally, Hand2 and Irx4 transcription factors have been shown to be reduced in SMYD1-deficient mice, suggesting that SMYD1-mediated histone methyla-
tion is necessary for the expression of these essential cardiac
transcription factors (89). Hence, these findings illustrate the
pervasive roles of histone methylation in the process of heart
development.
4. Related enzymes of histone lysine methylation and congenital heart disease

CHD is the most common type of birth defect, manifesting as obstacles in the process of embryonic heart or blood vessel development, which may result in the morphology, structure, function and metabolic abnormalities of heart and blood vessels (2,3,5). According to the statistics, CHD has become the first reason for birth defects and the main cause of perinatal death and death in children (2). The causes of CHD are not yet completely clear; however, most scholars consider that many types of CHD are caused by a single gene mutation and chromosome aberration, and most types of CHD belong to complex genetic diseases, which are caused by the interaction between genetic factors and environmental factors (90,91). Studies have shown that histone lysine methylation modification as part of the epigenetic regulation, is involved in the development of heart and blood vessels, which is also one of the causes of CHD (92,93). The level of histone lysine methylation is determined by the balance of histone methylation and demethylation, which is a process by which methyl groups are transferred onto or removed from the amino acids of histone proteins. Histone methyltransferase and histone demethylase catalyze histone methylation and demethylation, respectively. In most cases, under the action of the methylation and demethylation, the histone tails relax or surround, which can loosen or inhibit DNA of transcription factors so as to turn the genes in DNA 'off' and 'on', resulting in the normal or aberrant expression of related genes and leading to abnormal heart development. To understand the research progress of histone lysine methylation and CHD, we summarize the known histone lysine modifying enzymes which regulates CHD in Table I.

H3K4 methylation and CHD

Trithotax group (TrxG) proteins. During heart development, several cardiac progenitor pools give rise to diverse cell lineages, such as cardiomyocytes, vascular smooth muscle cells, fibroblasts that form the connective tissues and endothelial cells of the endocardium. The heart expresses many epigenetic factors, including both histone modifying proteins and chromatin remodelers. Among the epigenetic factors, TrxG proteins are special family of chromatin factors that regulate developmental gene expression in the heart (94). TrxG proteins function in multi-subunit complexes, three TrxG complexes, the MLL complex, the BRM/BAF complex and a supercomplex, and have been purified in mammalian cells (95). TrxG proteins are evolutionarily conserved H3K4 methyltransferases that maintain the transactivation states of lineage-specific genes during embryonic development. Multiple TrxG genes are normally expressed in the mouse heart. Due to the essential function of TrxG genes, constitutive knockouts of key TrxG genes often result in lethality during early embryogenesis before cardiac phenotypes can be analyzed. The differentiation of mouse embryonic stem cells (ESCs) toward mesodermal and endodermal lineages is severely altered and, in particular, the cardiac lineage differentiation of ESCs is completely abolished in the absence of MLL2, a TrxG member. Moreover, the expression of core cardiac transcription factors and the levels of H3K4 trimethylation of these cardiac-specific promoters are significantly decreased by the loss of MLL2 (96). Taken together, these results reveal a critical role for MLL2 in the proliferation and cardiac lineage differentiation of mouse ESCs, and provide critical insight not only into the novel role of the TrxG protein in cardiac development, but also into their clinical significance in related CHD.

SMYDs. SET and myeloid, nervy and DEAF-1 (MYND) domain-containing proteins (SMYDs), including SMYD1-5, have two functional protein domains, SET (mediates histone lysine methylation activity) and MYND (mediates the protein-protein interaction and binds to DNA motifs) domains (80). SET-MYND-domain 1 (SMYD1/BOP) encodes an evolutionary conserved histone methyltransferase containing a split SET domain interrupted by a MYND domain, which includes two members SMYD1a and SMYD1b, can catalyze H3K4 methylation (97). The expression of SMYD1 is restricted to skeletal and cardiac muscles in humans, fish, chickens and mice. There is evidence to indicate that SMYD1 plays important roles in cardiac differentiation, development and function (98). The global knockdown of SMYD1a and SMYD1b in zebrafish has been shown to result in the disruption of myofibril formation and an absence of beating of the heart. Molecular and cellular experiments showed that myofibers in embryos in which SMYD1 was knocked down appeared as immature myoblasts with centrally located nuclei and disorganized myofibrils, indicating that SMYD1 played a critical role in myofibers maturation and contraction (97). Conventional null SMYD1 mice die in utero around embryonic day 10.5 (E10.5) due to heart defects, including disrupted maturation of ventricular cardiomyocytes and malformation of the right ventricle (99). However, Diehl et al recently reported that SET and MYND domain containing 2 (SMYD2), is capable of H3K4 methylation when bound to Hsp90a and acts on non-histone targets by
Table I. Known histone lysine modifying enzymes involved in congenital heart diseases.

<table>
<thead>
<tr>
<th>Modifier of H3K4</th>
<th>Abnormal pattern</th>
<th>Species</th>
<th>Related cardiac phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trithohax group proteins</td>
<td>Deficiency</td>
<td>Mice</td>
<td>Embryonic lethal</td>
</tr>
<tr>
<td>MLL2</td>
<td>Deficiency</td>
<td>Mouse embryonic stem cells</td>
<td>Abnormal proliferation and cardiac lineage differentiation</td>
</tr>
<tr>
<td></td>
<td>Mutation</td>
<td>Humans</td>
<td>Atrial/ventricular septal defects, aortic coarctation</td>
</tr>
<tr>
<td>SMYDs</td>
<td>Deficiency</td>
<td>Zebrafish</td>
<td>Immature myofibers, non-beating heart</td>
</tr>
<tr>
<td>SMYD1</td>
<td>Deficiency</td>
<td>Mice</td>
<td>Embryonic lethal, hypoplastic right ventricle</td>
</tr>
<tr>
<td>SMYD3</td>
<td>Deficiency</td>
<td>Zebrafish</td>
<td>Pericardial edema, abnormal expression of heart-chamber markers</td>
</tr>
<tr>
<td>T-box transcription factors</td>
<td>Mutation</td>
<td>Humans</td>
<td>DiGeorge syndrome, double outlet right ventricle, ventricular septal defect</td>
</tr>
<tr>
<td>TBX1</td>
<td>Mutation</td>
<td>Humans</td>
<td>Ventricular septal defects</td>
</tr>
<tr>
<td>TBX2</td>
<td>Mutation</td>
<td>Mice, humans</td>
<td>Cardiac conduction dysplasia</td>
</tr>
<tr>
<td>TBX3</td>
<td>Mutation</td>
<td>Mice, humans</td>
<td>Defective epicardial and coronary blood vessel formation</td>
</tr>
<tr>
<td>TBX5</td>
<td>Mutation</td>
<td>Mice</td>
<td>Holt-Oram syndrome</td>
</tr>
<tr>
<td>TBX18</td>
<td>Mutation</td>
<td>Mice</td>
<td>Defective epicardium and coronary vessels</td>
</tr>
<tr>
<td>TBX20</td>
<td>Mutation/deficiency</td>
<td>Mice</td>
<td>Failed heart looping, defective chamber differentiation, cardiomyopathy, arrhythmias</td>
</tr>
<tr>
<td></td>
<td>Mutation</td>
<td>Humans</td>
<td>Septal defects, valvulogenesis defects, cardiomyopathy</td>
</tr>
<tr>
<td>DPF3</td>
<td>Mutation or deletion</td>
<td>Zebrafish</td>
<td>Incomplete cardiac looping, defective ventricular contractility and muscular fibers</td>
</tr>
<tr>
<td>PTIP</td>
<td>Deficiency</td>
<td>Mice</td>
<td>Abnormal cardiac conduction system, ventricular arrhythmia</td>
</tr>
<tr>
<td>SETD7</td>
<td>Mutation</td>
<td>Zebrafish</td>
<td>Developmental heart edema</td>
</tr>
<tr>
<td>LSD1</td>
<td>Mutation</td>
<td>Mice</td>
<td>Ventricular septal defects, salt-sensitive hypertension</td>
</tr>
<tr>
<td>BCOR</td>
<td>Mutation</td>
<td>Humans</td>
<td>Oculo-facio-cardio-dental (OFCD) syndrome</td>
</tr>
<tr>
<td>Modifiers of H3K9</td>
<td>Deficiency</td>
<td>Mice</td>
<td>Embryonic lethality, atrioventricular septal defects</td>
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<tr>
<td>G9a and GLP</td>
<td>Mutation/deficiency</td>
<td>Humans</td>
<td>Chromosome 9q subtelomere deletion syndrome with atrial/ventricular septal defect</td>
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<tr>
<td>Blimp-1/PRDM</td>
<td>Deficiency</td>
<td>Mouse</td>
<td>Ventricular septal defect and persistent arterial trunk</td>
</tr>
<tr>
<td>Jarid2</td>
<td>Mutation/deficiency</td>
<td>Mice</td>
<td>Ventricular/atrial septal defect, double-outlet right ventricle, dilated atra</td>
</tr>
<tr>
<td>Modifiers of H3K27</td>
<td>Mutation/deficiency</td>
<td>Mice</td>
<td>Bone dysplasia and heart development defects</td>
</tr>
<tr>
<td>Polycomb group proteins</td>
<td>Mutation/deficiency</td>
<td>Mice</td>
<td>Double outlet right ventricle, persistent truncus arteriosus, ventricular septal defects, atrial septal defects, atrioventricular canal defects and enlarged aortic valves, postnatal myocardial pathology</td>
</tr>
<tr>
<td>PRC1</td>
<td>Deficiency</td>
<td>Mouse embryonic stem cells</td>
<td>Embryonic lethality, reduced somite counts, heart malformation</td>
</tr>
<tr>
<td></td>
<td>Deficiency</td>
<td>Mouse embryonic stem cells</td>
<td>Failed to develop heart-like rhythmic contractions</td>
</tr>
<tr>
<td>UTX</td>
<td>Deficiency</td>
<td>Mouse</td>
<td>Embryonic lethality</td>
</tr>
<tr>
<td>Jmjd3</td>
<td>Deficiency</td>
<td>Mouse embryonic stem cells</td>
<td>Impaired mesoderm and subsequent endothelial and cardiac differentiation</td>
</tr>
<tr>
<td>Modifiers of H3K36</td>
<td>Mutation/deficiency</td>
<td>Humans</td>
<td>Sotos syndrome</td>
</tr>
<tr>
<td>NSD1</td>
<td>Deficiency</td>
<td>Mice</td>
<td>Perinatal lethal, atrial and ventricular septal defects</td>
</tr>
<tr>
<td>WHSC1</td>
<td>Mutation</td>
<td>Humans</td>
<td>Wolf-Hirschhorn syndrome</td>
</tr>
<tr>
<td>Jmjd5</td>
<td>Deficiency</td>
<td>Mice</td>
<td>Embryonic lethal</td>
</tr>
<tr>
<td>Modifiers of H3K79</td>
<td>Deficiency</td>
<td>Mice</td>
<td>Embryonic lethal, yolk sac angiogenesis defects, cardiac dilatation, cardiomyocyte death, systolic dysfunction, conduction abnormalities</td>
</tr>
<tr>
<td>DOT1L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modifiers of H4K20</td>
<td>Mutation/deficiency</td>
<td>Humans</td>
<td>Sotos syndrome, ventricular/atrial septal defect, patent ductus arteriosus</td>
</tr>
</tbody>
</table>
inhibiting the functional activity of p53 via methylation of p53, lysine 370, which was differentially expressed during cardiac development with highest expression in the neonatal heart (100). To elucidate the functional role of SMYD2 in the heart, they generated knockout mice harboring a cardiomyocyte-specific deletion of SMYD2 and performed histological, functional and molecular experiments. Unexpectedly, cardiac deletion of SMYD2 was dispensable for proper morphological and functional development of the murine heart (100). H3K4 methyltransferase SMYD3 is highly expressed within developing zebrafish heart and knockdown of it led to severe defects such as pericardial edema and abnormal expression of three heart-chamber markers in cardiac morphogenesis (101). These results indicate that SMYD3 plays an important role in heart development and its proper functioning is essential for normal heart morphogenesis during development.

**T-box (TBX) transcription factors.** TBX transcription factors share a highly conserved DNA-binding domain and play critical roles in embryonic development (102). Six members of TBX family (TBX1, TBX18 and TBX20 of the TBX1 subfamily, and TBX2, TBX3 and TBX5 of the TBX2 subfamily) are required for the cardiac morphogenesis in mammals (103). TBX1 interacts with H3K4 methyltransferase to enhance its H3K4 monomethylation status through T-box, regulates expression of related genes by epigenetic patterns (104). TBX1 mutation can lead to DiGeorge syndrome (DGS), which is the most common microdeletion syndrome, and is characterized by congenital cardiac, craniofacial and immune system abnormalities (105). Additionally, Pan et al reported that a novel heterozygous TBX1 mutation, p.Q277X, was identified in an index patient with double outlet right ventricle and ventricular septal defect (106). TBX2 gene is expressed in the myocardium of the atrioventricular canal, outflow tract and inflow tract and plays a critical role in heart chamber formation (107). The genomic deletion and duplication of TBX2 gene have been found to be associated with ventricular septal defects (108). The evolutionary conserved TBX3 gene encodes T-box transcription factors and locus forms a CTCF independent autonomous regulatory domain with multiple combinatorial regulatory elements, which plays crucial roles in the development and homeostasis of the cardiac conduction system in humans and mice (109). Previous studies have found that TBX5 is expressed in the proepicardial organ or septum transversum, which is required for the normal development of proepicardium/proepicardial organ cells, as well as proper epicardial formation and maturation (110). Additionally, TBX5 deficiency delays epicardial cell attachment to the myocardium and impairs production of epicardial-derived cells and their migration into the myocardium, and results in abnormal coronary vasculogenesis and murine ischemic cardiomyopathy (111). Clinical studies have shown that Holt-Oram syndrome is caused by mutations in TBX5, which is a human inherited disorder and manifests as left pericardium agenesis and anomalous coronary arteries along with ventricular septal defects (112-114). These findings all demonstrate that TBX5 is essential for epicardial development in hearts and establishment of the coronary vasculature. Similar to TBX5, TBX18 is also highly expressed in proepicardial cells and proepicardium, TBX18-deficient proepicardium produces an epicardium and coronary vasculature with structural and functional defects, and that remodeling of the disorganized subepicardial plexus in TBX18-deficient hearts produced a mature coronary artery network with fewer distributing conduit vessels and smaller lumen profiles, which indicates that TBX18 plays critical role in coronary development (115). However, TBX20 is necessary in heart development by regulating cardiomyocyte proliferation and regional specification and formation of cardiac chambers and valves; TBX20 mutations in mice can result in the failure of heart looping, developmental arrest, and the lack of chamber differentiation, and loss of TBX20 in mice leads to cardiomyopathy with associated arrhythmias and death (116,117). More seriously, mutations in human TBX20 result in cardiac malformations including septal defects, double outlet right ventricle and cardiomyopathy (118,119). These findings provide novel insight into the molecular mechanism underlying CHD and suggest potential implications for the development of novel preventive and therapeutic strategies for CHD.

**DPF3.** DPF3 is a member of the highly conserved d4 protein family, which is characterized by a double PHD finger in the C-terminal and has two splice variants DPF3a and DPF3b in human and mice (120). In the process of embryonic development, DPF3 is expressed in both heart and somites of mouse, chicken and zebrafish, which is important epigenetic regulation factor for heart and muscle development by associated with the BAF chromatin remodeling complex and binds methylated lysine residues of H3K4 (121). Previous studies have found that DPF3 mutation or deletion leads to incomplete cardiac looping, attenuated ventricular contractility and disassembled muscular fibers caused by the transcriptional deregulation of structural and regulatory proteins in the heart, which all demonstrate that DPF3 is responsible for cardiac development imbalance, ventricular septal defect and other cardiac disorders (121).

**Pax transactivation domain interacting protein (PTIP).** PTIP is an essential cofactor for H3K4me by KMT2C/D, which is encoded by the Paxip1 gene and is essential for embryonic development in mice and flies (122-124). As a critical component of the KMT2C/D complex, the loss of PTIP leads to reduced levels of H3K4me3 in whole embryos, ESCs and Drosophila larvae (125,126). Stein et al demonstrated that temporal and tissue-specific deletion of PTIP reduces H3K4 methylation level and alters the transcriptional program in nondividing cardiomyocytes. It is suggested that a role for KMT2 complexes not just in establishing active chromatin domains but also in the maintenance of the differentiated state over time. Furthermore, the loss of PTIP-mediated H3K4me results in significant changes in the physiology of the cardiomyocytes, suggesting that PTIP deletion is the direct cause of premature ventricular beats, a harbinger of lethal ventricular arrhythmias in nondividing cardiomyocytes (42,127).

**SET domain containing protein 7 (SETD7).** SETD7 also termed as SET7/9, is another type of histone lysine methyltransferase and only has SET domain for methyltransferase activity, but not MYND domain, which is initially discovered as a specific methyltransferase for nonmethylated H3K4. Tao et al found that the knockdown of SETD7 showed the defects in skeletal muscle formation and myofibril structures in a zebrafish developmental model (128). To examine the function of SETD7 in heart development, Kim et al firstly demonstrated that SETD7 was highly expressed in developing zebrafish heart and knockdown of it led to severe defects in cardiac morphogenesis such as developmental heart edema.
Furthermore, the double knockdown of SMYD3 and SETD7 caused synergistic defects in heart development. Similar to the knockdown effect, the overexpression of SETD7 also caused the heart morphogenesis defects in zebrafish (85). These results indicate that the histone modifying enzyme, SETD7, plays an important role during heart development and its proper functioning is essential for normal heart morphogenesis during development.

Lysine-specific demethylase 1 (LSD1). LSD1 (also known as AOF2/KDM1A), is a member of a group of enzymes with lysine specific demethylase activity. LSD1 performs enzymatic activity toward di- and monomethyl H3K4 and H3K9 respectively; the specificity for H3K9 arises when LSD1 binds to the androgen receptor, resulting in a shift of its activity from H3K4 (129). LSD1 interacts with proteins mostly through the tower domain, an extended helical structure. Furthermore, there is evidence to indicate that LSD1-interacting proteins can regulate the activity and specificity of LSD1 in developmental processes (130,131). Nicholson et al found that mice homozygous for a hypomorphic LSD1 allele exhibit a failure to survive after birth perinatally due to heart defects, with the majority of animals suffering from ventricular septal defects (132). Therefore, the above-mentioned studies thereby illuminate a novel role for LSD1 in the development of the mammalian heart.

BCL-6 corepressor (BCOR). It was found that BCOR inhibited gene transcription by interacting with BCL-6, and BCOR mutation resulted in abnormal activation of AP-2a, which was a key factor that mediated the differentiation of bone marrow mesenchymal stem cells (MSCs) (133). Fan et al also pointed out that BCOR recruited a histone demethylase JHDM1B to the target gene promoter, resulting in the demethylation of H3K4me3 and H3K36me2 and transcription repression of genes; however, BCL-6 mutation may impair the recruitment of JHDM1B to chromatin, resulted in increased methylation levels of H3K4 and H3K36 (133). Abnormal histone methylation due to BCOR mutation may affect BCL-6 binding to the AP-2a promoter, causing aberrant activation of gene and resulting in the in occurrence of oculo-facio-cardio-dental (OFCD) syndrome, which is a rare genetic disorder characterized by teeth with extremely long roots, and craniofacial, eye and congenital cardiac abnormalities include septal defect and mitral valve defect abnormalities (133-135). On the whole, it was identified that BCOR mutation affected heart development and AP-2a played a role in congenital heart defects associated with OFCD patients, and indicated that BCOR may be a novel target for diagnostic and treatment strategies of OFCD syndrome.

H3K9 methylation and CHD

G9a and GLP. G9a and GLP are known as major H3K9 mono- and di-methyltransferases and contribute to transcriptional silencing, which play critical biological roles in various cells and tissues. For example, G9a and GLP are indispensable for mouse early development; G9a or GLP knockout mice exhibit embryonic lethality around E9.5 due to severe growth defects (38,136,137). In order to clarify the roles of G9a and GLP in cardiac development, Inagawa et al analyzed the phenotypes of cardiomyocyte specific GLP knockout and G9a knockdown mice, it was shown that the H3K9me2 level decreased mark-
functions important for transcriptional repression. In mammals, 2 major Polycomb group complexes exist: polycomb repressive complex 1 (PRC1) and PRC2. Whereas PRC1 ubiquitinitates histone H2A on Lys119, PRC2 catalyzes dimethylation and trimethylation of H3K27, generating H3K27me2/3 (147). Weston et al pointed out that Rae28 protein, the core component of PRC1, which made PRC1 bind to H3K27me3 and then formed chromatin tight structure to prevent the occurrence of transcription. Rae28 mutation or deletion mice tend to perform bone dysplasia and heart development defects (148). However, EzH2, the major histone methyltransferase of PRC2, trimethylates H3K27 and is essential for embryonic development (149). Delgado-Olguin et al have shown that EzH2 stabilizes cardiac gene expression and prevents cardiac pathology, but EzH2 deletion in cardiac progenitors causes postnatal myocardial pathology and destabilizes cardiac gene expression, which suggests that EzH2 is essential for stable postnatal heart gene expression and homeostasis (150). Furthermore, Chen et al demonstrated that a variety of cardiovascular structural malformations were observed in the EzH2 mutant mice, including double outlet right ventricle, persistent truncus arteriosus, membranous and muscular ventricular septal defects, atrial septal defects, atrioventricular canal defects and enlarged aortic valves, which defined an indispensable role of EzH2 in normal cardiovascular development (151).

Ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX). Histone demethylase UTX, also known as KDM6A, that specifically targets the repressive H3K27me3 modification plays an important role in the activation of ‘bivalent’ genes in response to specific developmental cues. Welstead et al showed that UTX-deficient embryos had reduced somite counts, neural tube closure defects and heart malformation that presented between E9.5 and E13.5 (152). Other studies have also found that UTX-deficient ESCs failed to develop heart-like rhythmic contractions under a cardiac differentiation condition; UTX deficient mice exhibited severe defects in heart development and embryonic lethality; these data establish that UTX is required for heart development acts as a critical switch to activate the cardiac developmental program (153,154).

Jumonji domain-containing protein 3 (Jmjd3). Jmjd3 (KDM6B), another H3K27 demethylase, functions redundantly with UTX. Jmjd3 is induced and participates in Hox gene expression during development, neuronal differentiation and inflammation, and recent data suggest that Jmjd3 inhibits reprogramming by inducing cellular senescence (155). Jmjd3 deficient mice showed embryonic lethality before E6.5, suggesting a crucial role of Jmjd3 in early embryonic development (156,157). The ablation of Jmjd3 in mouse ESCs impaired mesoderm and subsequent endothelial and cardiac differentiation. These results clarify that Jmjd3 is necessary for mesoderm differentiation and cardiovascular lineage commitment (158).

H3K27 methylation and CHD. H3K27 methylation is related to gene activation and DNA damage repair. Histone methylation occurs at H3K27 is catalyzed by yeast disruptor of telomeric silencing (DOT1) and its mammalian homolog, DOT1L. DOT1 is a kind of evolutionarily highly conservative histone methyltransferase, which does not contain the SET domain structure, can be specific to different methylation levels in the H3K79. Compared with other histone lysine methylation, in yeast, DOT1 activity is positively regulated during transcription elongation through Rad6-Bre1 mono-ubiquitination of H2B (166). Recently, loss-of-function experiments revealed a critical role of DOT1L during mouse embryogenesis, as germline Dot1L knockout caused lethality at E10.5 with growth impairment, yolk sac angiogenesis defects, and cardiac dilation (167). In addition, cardiac-specific knockout of DOT1L resulted in increased mortality rate with chamber dilation, increased cardiomyocyte cell death, systolic dysfunction and conduction abnormalities (168). These phenotypes mimic those exhibited in patients with dilated cardiomyopathy. Interestingly, Nguyen et al demonstrated that DOT1L is downregulated in idiopathic DCM patient samples compared with normal controls (168). Therefore, the above studies not only establish
a critical role for DOT1L-mediated H3K79 methylation in cardiomyocyte function, but also open new avenues for the diagnosis and treatment of CHD.

**H4K20 methylation and CHD.** H4K20 can be catalyzed to different forms of monomethylation, dimethylation and trimethylation, PR-SET7 can only single methylate H4K20, but double and triple methylation of H4K20 are catalyzed by two other methyltransferases SUV4-20h1 and SUV4-20h2 (169). It is shown that H4K20 methylation is related to transcription silence, H4K20me3 plays a vital role in the regulation of DNA damage but not directly regulates the expression of genes (170). In addition, Tatton-Brown and Rahman have found that NSD1 is a protein containing the SET domain structure, which with specific H4K20 and H3K36 methyltransferase activity (171). NSD1 mutation or deficiency is the main cause of Sotos syndrome which with a high incidence of CHD characterized by ventricular septal defect, atrial septal defects and patent ductus arteriosus (159,160).

5. Conclusion and prospection

Histone modification is the important content of epigenetics, which is not only showed as directly regulating gene expressions, but also influencing gene activity through DNA modification because of its intimate touching with DNA. However, single histone modification usually cannot come into effect individually, and it determines together the gene expression of genome through collaborative effect of multiple histone modifications; but, yet so far, about the mechanism of histone modification, especially the specific mechanism of regulation of histone modification is not quite clear. Therefore, although the histone lysine methylation modification is related to CHD, further intensive research is still needed to illuminate relationship at the level of molecule. At the same time, besides the genetic factors of CHD pathogenesis, there still exist outer environmental factors, so that the complexity of diagnosing and treatment of diseases has been increased. However, it is clear that the comprehensive and meticulous investigation of histone lysine methylation modifications may provide new insight and understanding into the exploration of CHD pathogenesis and targeted prevention.

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