

Chapter 3

The Emerging Role of Epigenetics



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Abstract Epigenetics is one of the most rapidly expanding fields in biology over the past decades. Epigenetic mechanisms, including DNA methylation, histone modifications, and RNA-associated editing, lead to the heritable silencing/activation of genes without changes in DNA sequence. The critical role of epigenetic modifications has been demonstrated in normal and disease development in humans. With the advent of next-generation sequencing, the technological breakthrough makes it possible to unveil the genome-wide mapping of epigenetic changes. Here, we give a comprehensive overview of epigenetic mechanisms and focus on the recent progress of epigenetic modifications involved in the pathogenesis or progression of human diseases, in particular, cardiovascular diseases and cancers. In addition, some current epigenetic therapies including the inhibitors of DNA methyltransferases and histone deacetylases that have shown promising therapeutic effects will be also discussed.

Keywords Epigenetics · DNA methylation · Histone modification · miRNAs and lncRNAs

Abbreviations

3' UTR	3' untranslated region
5-aza-dC	5-aza-2'-deoxycytidine
CADs	Coronary artery diseases
ChIP	Chromatin immunoprecipitation
CVDs	Cardiovascular diseases
DCM	Dilated cardiomyopathies

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DNMTs	DNA methyltransferases
eNOS	Endothelial nitric oxide synthase
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
HF	Heart failure
iNOS	Inducible nitric oxide synthase
lncRNAs	Long noncoding RNAs
MBDs	Methyl-CpG-binding proteins
miRNAs	microRNAs
NGS	Next-generation sequencing
NO	Nitric oxide
PAH	Pulmonary arterial hypertension
SMCs	Smooth muscle cells
TSGs	Tumor suppressor genes

1 Introduction

1.1 Cardiovascular Diseases

Cardiovascular diseases (CVDs) are the worldwide leading cause of human morbidity and mortality. CVDs encompass a series of pathological conditions, such as hypertension, coronary artery diseases (CADs), myocardial infarction (MI), and chronic heart failure (CHF). CHF is the final stage of most CVDs; however, recent pharmacotherapy on CHF management is limited to diuretics, renin-angiotensin-aldosterone (RAA) inhibitors, and vasodilators, targeting blood pressure, vasodilation, cardiac contractility, and hypertrophy (Swedberg et al. 2005). Despite efforts in revitalizing therapeutic management over decades, mortality remains unacceptably high with 50% of survival rate 5 years after diagnosis (Roger 2013). Moreover, after the process of thrombosis and coronary atherosclerosis, CAD could evolve into myocardial ischemia by plaque rupture or erosion (Ambrose et al. 1988). CAD is another leading cause of morbidity and mortality affecting the cardiovascular systems in developed countries. Therefore, in addition to the known mechanisms and therapies, there is clearly an unmet need for the exploration of better therapeutic targets for CVDs.

1.2 Epigenetics

The word “epigenesis” was coined by Conrad Waddington in the twentieth century to link the fields of developmental biology and genetics, which is broadly defined as how genotypes give rise to phenotypes during development (Waddington 1952). Several decades later, the term “epigenetics” was proposed as the study of

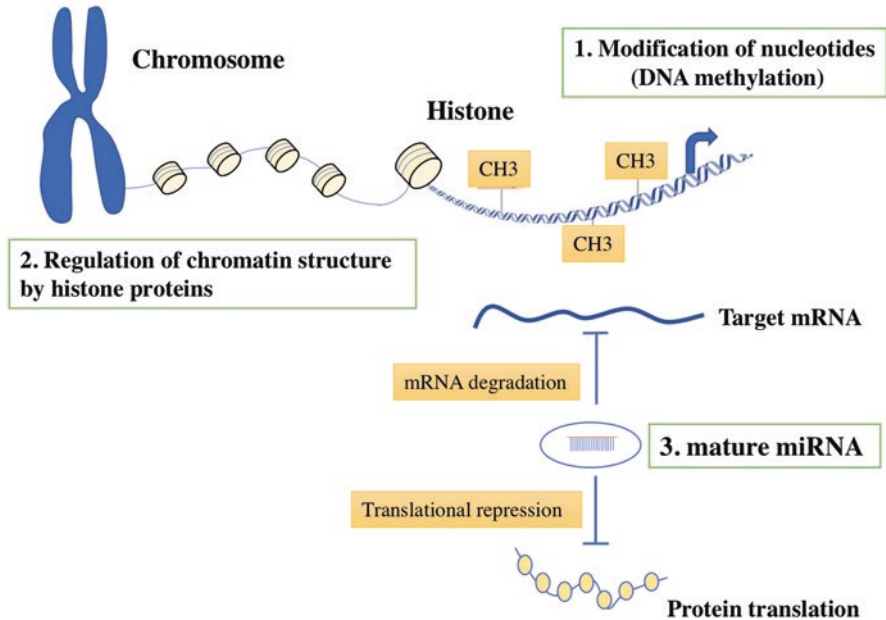


Fig. 3.1 Mechanisms of epigenetic modifications: DNA methylation, histone modification, and RNA-associated silencing

mitotically and meiotically heritable changes in gene function that are not coded in DNA sequence itself (Riggs 1975; Razin and Riggs 1980). Epigenetics explores the mechanisms that make cells adapt quickly to environmental changes and build a bridge between the environment and genes. The mechanisms of heritable epigenetics have been extensively studied in different organisms and encompassed an array of molecular modifications to both DNA and chromatin, including DNA methylation, histone modification, and RNA-associated silencing, which are used to initiate and sustain epigenetic silencing (Egger et al. 2004) (Fig. 3.1). These modulators could modify the binding of transcription activators and repressors with respective gene promoters and/or change the function and conformation of chromatin, further changing the expression and function of some key genes implicated in specific signaling pathways (Gluckman et al. 2009). In general, compared with the short and flexible effect of histone modifications, DNA methylation seems to work as long-term silencers of gene expression, in which the cross talk between them exists in different mechanisms (Goldberg et al. 2007). Furthermore, the function of epigenetics has been extensively evaluated in human cancers, and recent studies have started to explore the research field of epigenetic role implicated in the development and progression of CVDs (Turunen et al. 2009; Loscalzo and Handy 2014).

1.3 DNA Methylation

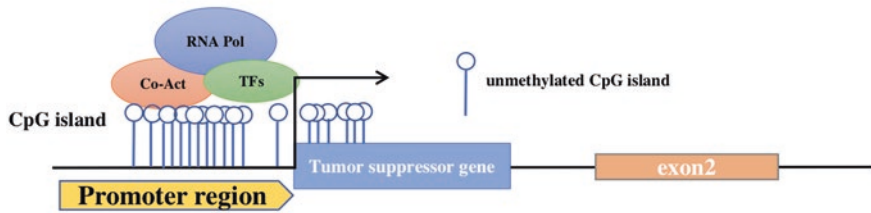
DNA methylation was proposed as the first crucial epigenetic mechanism for the switching and heritability of gene activities (Griffith and Mahler 1969; Holliday and Pugh 1975). As the most widely studied epigenetic modification, DNA methylation is defined as the addition of a methyl group (-CH₃) to the carbon 5 position of cytosine ring in a CpG dinucleotide by DNA methyltransferases (DNMTs) (Jones 2001; Robertson 2005). The DNA methylation machinery is made up of two parts, DNMTs, which establish and maintain DNA methylation patterns, and the methyl-CpG-binding proteins (MBDs) family, which are candidates for the readout of DNA methylation (Robertson 2005).

MBD family is composed of five well-characterized proteins, including MeCP2, MBD1, MBD2, MBD3, and MBD4, of which, at least MBD1, MBD2, and MeCP2 could directly repress transcription (Ng et al. 1999; Ballestar and Esteller 2005). Methylation of the promoter CpG island results in the recruitment and binding of MBDs and transcription repressors including histone deacetylases (HDACs) and Sin3A, which further leads to the blockage of transcription initiation (Jones et al. 1998; Ng, Zhang et al. 1999). On the contrary, unmethylated promoters commonly lack MBD proteins with the exception of MBD1 (Lopez-Serra et al. 2006). Upon the treatment of demethylating agent, the promoter CpG islands of cancer cells will be hypomethylated and accompanied by MBD protein release and gene re-expression, suggesting the important association of MBD proteins and methylation status.

DNMT1, DNMT3a, and DNMT3b have the transcription repressor domains which can recruit HDACs and/or other corepressor proteins to DNA in a way similar to MBDs (Rountree et al. 2000; Bachman et al. 2001). Of the three DNMTs, DNMT1 is required for maintaining patterns of methylation, while DNMT3a and DNMT3b are responsible for de novo methylation and mammalian development (Okano et al. 1999; Branco et al. 2008). The maintenance DNMT1 is most abundant in the cells, which is needed to methylate hemimethylated sites that are generated during semiconservative DNA replication (Jones and Liang 2009; Portela and Esteller 2010). Different from DNMT1, DNMT3A and DNMT3B are highly expressed in embryonic stem cells and downregulated in differential cells, which might serve to establish the pattern of methylation during embryonic development (Esteller 2007). Methylation gives some explanations to phenomena in many cellular processes, such as gene imprinting or X chromosome inactivation in female mammals, both of which can be stably maintained (Reik and Walter 2001; Huynh and Lee 2005).

In normal tissues, DNA methylation occurs in 80% of repetitive DNA regions, including tandem repeats (such as satellite DNA) and dispersed repeats (such as long interspersed transposable elements (LINEs), short interspersed transposable elements (SINEs), and endogenous retroviruses) (Yoder et al. 1997; Robertson 2005). CpG islands are small concentrated regions of CpG-rich dinucleotides with the length of 0.5 to several Kb, which show elevated G+C base composition (Bird

1. Normal cell-unmethylated CpG island



2. Cancer cell-methylated CpG island

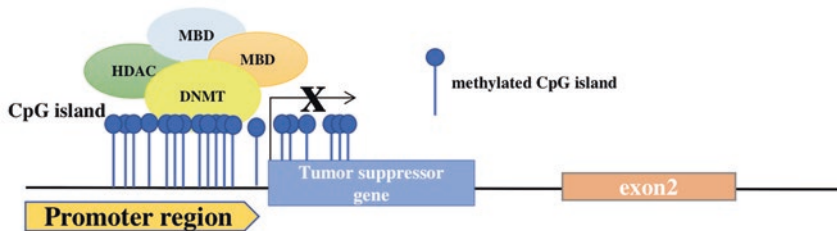


Fig. 3.2 DNA methylation patterns in normal and cancer cells

1986). The frequency of CpG dinucleotides in human genome is lower than expected; however, approximately 70% of human gene promoters contain CpG islands, whereas some CpG islands are found within the body of genes or even 3' region (Saxonov et al. 2006). The majority of these promoters are associated with housekeeping genes, as well as half of tissue-specific genes and development regulatory genes (Bird 1986; Larsen et al. 1992).

DNA methylation of these promoter CpG islands correlates with transcriptional repression in mammalian cells (Goll and Bestor 2005) (Fig. 3.2). Normally, the CpG islands associated with the promoters are usually unmethylated, leading to the transcription of the gene. The importance of DNA methylation for inhibition of gene expression and transcriptional silencing is apparent in the exceptions of gene imprinting or X chromosome inactivation in females (Herman and Baylin 2003). The fully methylated CpG islands leading to global gene silencing on X chromosomes and the silenced alleles of some imprinted genes are considered as a form to distinguish maternal and parental determination which are programmed in condition that only one allele of the gene is expressed in normal tissues (Antequera and Bird 1993; Bird 2002; Reik and Lewis 2005). Genomic proper patterns of methylation in mammals are essential for gene regulation and chromatin organization during embryogenesis (Goll and Bestor 2005).

1.4 Histone Modifications

Chromatin is the complex of DNA and proteins that are packaged within the nucleus of eukaryotic cells. Histones are a family of those small proteins that facilitate DNA to condense into chromatin. Importantly, the acetylation and methylation of conserved lysine residues in histone tail domain are known as the posttranslational modifications of histones. Histone acetylation is the best-understood histone modification. Of note, the critical role of acetylation in chromatin structure regulation and gene expression has been recently demonstrated (Cheung et al. 2000; Yang and Seto 2007). Most well-characterized acetylation substrates are nucleus-localized transcription factors and its coregulatory machinery (Blander and Guarente 2004; Yang and Seto 2008). These transcription repressors and activators recruit HDACs and histone acetyltransferase (HAT), respectively. Largely, HDACs lead to transcriptional inhibition by removing acetyl group, and the HATs work to increase the transcription by adding the acetyl group to the conserved lysine residue on N-terminal tail of the histone proteins (Brown et al. 2000).

HDAC Recent evidence suggests that many HDACs consist of highly conserved domain and are subdivided into four major classes in mammals.

1. Class I HDACs (HDAC1, 2, 3, and 8) comprise of domain surrounded by short NH₂ and COOH termini and are widely expressed.
2. Class II HDACs contain one or two catalytic sites and are further subdivided into two subclasses, including subclass IIa (HDAC4, 5, 7, and 9) and subclass IIb (HDAC6 and 10) (Yang and Seto 2008).
3. Class III HDACs are sirtuins (SIRT1-SIRT7).
4. Class IV is known to be homologs of Hda1 and Rpd3 protein of yeast and consists of a solitary membrane HDAC11 (Majumdar et al. 2012; Wang et al. 2014).

HAT There are two subcategories of HATs including nuclear and cytoplasmic, which are designated as type A HATs and type B HATs, respectively (Thiagarajan et al. 2016).

Type A is transcription related and further divided into five families.

1. GNAT-related acetyltransferase family represented by general control nonderepressible (GCN5), p300/CBP-associated factor (PCAF), and elongator complex protein 3 (ELP3).
2. p300/CBP family represented by p300 and CBP.
3. MYST family consists of MYST1 (HMOF, males absent on the first), MYST2 (HBO1, histone acetyltransferase binding to ORC), MYST3 (MOZ, monocytic leukemia zinc finger), MYST4 (MORF, monocytic leukemia zinc finger protein-related factor), and TIP60 (tat interacting protein 60 kDa).
4. Basal TF family: TFIIC (transcription factor IIIC), TAF1.
5. Nuclear receptor cofactor (NRCF) family, steroid receptor coactivator (SRC), ACTR/NCOA3 (nuclear receptor coactivator 3).

Type B HATs such as HAT1, HAT2, Hat B3.1, and Rtt109 are relatively characterized.

Briefly, histone acetylation can modify gene transcription at both global acetylation level and promoter-specific acetylation level (Gottesfeld and Forbes 1997; Vaissière et al. 2008). More importantly, there is evidence showing the intimate cross talk between histone modification and DNA methylation in the process of gene silencing. The loss of histone acetylation may initiate gene silencing, which is followed by the induction of DNA methylation (Daujat et al. 2002; Vaissière et al. 2008).

1.5 *Noncoding RNAs*

Recently, the discovery of the noncoding RNAs (ncRNAs) provided further evidence to posttranslational mechanisms defined as epigenetic changes. It has become apparent that noncoding portion of the genome is of great importance for normal development, differentiation, and multiple diseases (Friedman and Jones 2009; Esteller 2011). Those ncRNAs involved in epigenetic processes could be categorized into the short ncRNAs (<30 nucleotides (nt)) and the long noncoding RNAs (lncRNAs) (>200 nts). The three major classes of short ncRNAs are microRNAs (miRNAs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs) (Kowalczyk et al. 2012). Both major groups are found to implicate in a wide range of cellular processes, heterochromatin formation, histone modification, and transcriptional silencing (Grewal and Moazed 2003; Moazed 2009). Here, we mainly focus on the epigenetic role of miRNAs and lncRNAs.

miRNAs belong to a class of single-stranded, small noncoding RNAs of 19–25 nts in length. miRNAs are transcribed from long RNA precursors (pri-miRNAs) by polymerase II and stabilized by 5' capping and 3' polyadenylation (Bartel 2009) (Fig. 3.3). The hairpin secondary structure of pri-miRNA is recognized and processed by the microprocessor complex (RNase III enzyme Drosha and DGCR8/Pasha) to release 60–80 nts pre-miRNA (Lee et al. 2003; Denli et al. 2004; Gregory et al. 2004). A pre-miRNA of the hairpin or stem-loop structure is exported into cytoplasm by Exportin 5 and further cleaved by another RNase III enzyme Dicer to generate the mature miRNA (Han et al. 2004; Kim 2005). Mature miRNAs result in translational repression or mRNA degradation of the target protein-coding genes through imperfect base pairing with the 3' untranslated region (3' UTR) of targeted mRNAs (Bartel 2004).

On the other hand, with the advent of high-throughput genome studies, a new class of lncRNAs measuring longer than 200 nts in length without evident protein-coding function was discovered (Martin et al. 2012). As a broad definition, lncRNA is encompassed by different classes of RNA transcripts, such as small nucleolar RNA (snoRNA), enhancer RNAs, and intergenic transcripts (Batista and Chang 2013). lncRNAs could regulate gene expression by modification of accessibility of gene promoters, which is further facilitated to tune the gene expression when

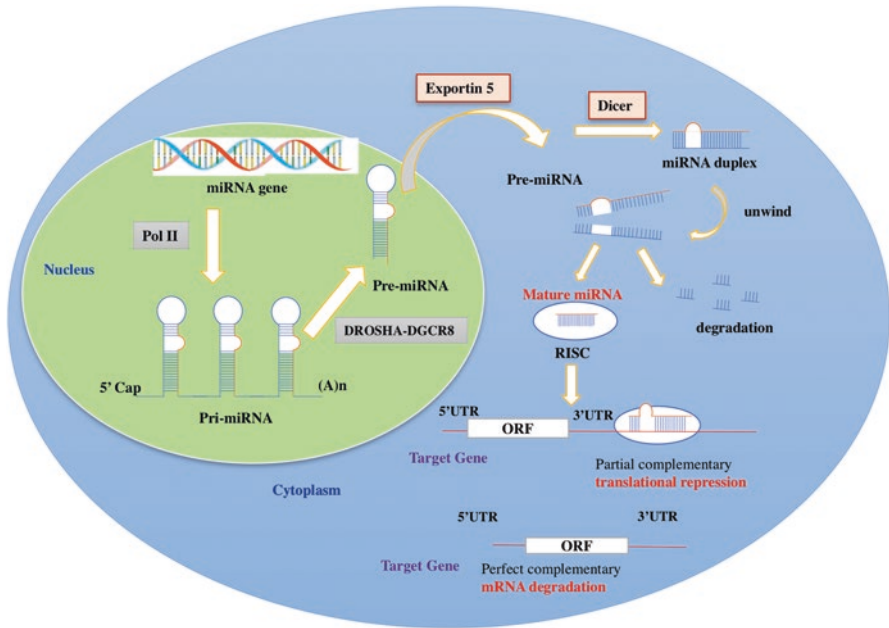


Fig. 3.3 miRNA biogenesis and function

adapting to the changes of environmental conditions or silence a gene during the developmental program (Batista and Chang 2013; Kornienko et al. 2013). Multiple studies have shown that the expression of miRNAs and lncRNAs is more tissue or cell specific than that of protein-coding genes (Lagos-Quintana et al. 2002; Ravasi et al. 2006; Djebali et al. 2012).

2 Epigenetics and Diseases

Gene expression patterns in different cell types should be appropriately maintained responding to environmental and developmental changes, whereas inappropriate expression might result in diseases. The important association of epigenetics with diseases is addressed by the increasing number of human diseases that are known to happen when the epigenetic information is not correctly maintained. It has become clear that aberrant regulation of DNA methylation, miRNAs, and lncRNAs contributes to the initiation or progression of human diseases (Robertson 2005; Calin and Croce 2006; Brunner et al. 2012). Further interests in this field come from the evidence of the interaction and cross talk among DNA methylation, histone modification, and small RNAs, as well as the developing pharmacological ways of reversing epigenetic abnormalities (Brown and Strathdee 2002; Tsai et al. 2010).

2.1 DNA Methylation and Diseases

2.1.1 DNA Methylation and Cancer

A large group of diseases are linked to the abnormalities of DNA methylation, showing the aberrant DNA methylation patterns and the pathological impact of their dysregulation in repeat instability. Among all diseases, the best-known studied disease is cancer. The association of methylation and cancer was firstly reported in 1983, which has been shown that cancer cells are hypomethylated compared with normal counterparts (Feinberg and Vogelstein 1983). Loss of methylation is an early and frequent event in carcinogenesis, which is generally more pronounced with cancer severity and metastatic potential in different tumor types (Widschwendter et al. 2004). For instance, age-dependent hypomethylation of specific DNA sequence was found to precede diploidy loss in a subset of gastrointestinal cancers (Suzuki et al. 2006). On the other hand, hypomethylation in cancer cells may result in the activation of oncogenes, further leading to the genomic rearrangements and instabilities (Herman and Baylin 2003). The synuclein- γ gene seems to be a target of oncogenic effect, demethylation of which serves as a molecular indicator of metastasis in a large group of human cancers, including breast cancer, ovarian cancer, and hepatocellular cancer (Gupta et al. 2003; Zhao et al. 2006).

In contrast to oncogene, biallelic loss of function in tumor suppressor genes (TSGs) is responsible for tumorigenesis as described by the Knudson's hypothesis. This hypothesis was derived from families with hereditary cancers, in which children who suffer an inactivating germ line mutation of one allele and later a second somatic mutation of the other allele have the chance to predispose to cancer formation (Knudson 1997). Of note, essential TSGs are found to be mapped to frequent regions of homozygous deletion or loss of heterozygosity (LOH) in some human cancers, such as CDKN2A mutation in melanoma, RB1 mutation in retinoblastoma, and TP53 inactivation in Li-Fraumeni syndrome (Malkin 1993; Monzon et al. 1998; Thiagalingam et al. 2002; Wang and Chim 2015). Given that DNA methylation results in gene silencing, promoter methylation of TSGs may work as a second hit in addition to gene mutation or deletion, to fulfill the Knudson's hypothesis in carcinogenesis (Wang and Chim 2015). Global DNA hypomethylation but gene-specific hypermethylation of promoter-associated CpG islands is a hallmark of human cancers (Esteller 2007). Interestingly, promoter CpG island methylation of TSGs has been noted in a variety of human cancers. In addition to gene silencing and loss of classic tumor suppressor function, it could also affect different cellular signaling pathways with tumor-specific profile and miRNAs with growth inhibitory role (Esteller 2007; Wang and Chim 2015). For instance, a diverse group of TSGs has been reported to be methylated in solid tumors and hematological cancers, suggesting the implication of hypermethylation in the carcinogenesis, including CDKN2A/CDKN2B regulating cell cycle in ependymal tumors, SFRP family (SFRP1/SFRP2/SFRP3/SFRP5) regulating WNT signaling in colorectal cancer and acute myeloid leukemia, and SOCS1/SOCS3/SHP1 regulating the JAK/STAT

signaling pathways in hepatocellular carcinoma and multiple myeloma (Yoshikawa et al. 2001; Rousseau et al. 2003; Chim et al. 2004; Suzuki et al. 2004; Niwa et al. 2005; Valencia et al. 2009).

The genome-wide DNA hypomethylation is a consistent finding in human tumors; however, the importance and the detailed mechanism of this change remain an open question. Several animal models are used for studying the effects of changes in DNA hypomethylation implicated in carcinogenesis. Lin et al. investigated the effect of conditional inactivation of de novo methyltransferase DNMT3b in *Apc^{Min/+}* mice. The results support the role of DNMT3b in the transition stage between microadenoma formation and macroscopic colonic tumor growth, and suggest the link of DNA hypomethylation and decreased progression of microadenoma to macroscopic tumor growth (Lin et al. 2006). On the other hand, several studies show the important function of maintenance DNA methyltransferase DNMT1 involved in tumor formation. DNA hypomethylation in the transgenic *Apc^{Min/+}* mice containing a mutant DNMT1 is shown to promote early formation of hepatocellular adenomas and carcinomas but suppress overall progression of tumorigenesis, indicating the opposite effects of DNA hypomethylation in different cell types (Yamada et al. 2005). Mice carrying a hypomorphic allele of DNMT1 (*DNMT1^{chip/-}*) developed thymic lymphomas with the generation of intracisternal A particle (IAP) somatic insertion in the middle of the oncogenic *Notch1* genomic locus, demonstrating that DNA hypomethylation could induce tumors accompanied by the activation of endogenous retroviral elements and thus the chromosomal instabilities (Howard et al. 2008). Therefore, the different outcomes of animal models may vary from different types of cells, tissues, and tumors that are studied, the background of the animals, as well as the specific DNA sequences and chromosomal variations (Song et al. 2005; Yamada et al. 2005; Illingworth et al. 2008).

2.1.2 DNA Methylation and Atherosclerosis

The role of methylation involved in CVDs remains largely unknown. It has been noted that loss of DNA methylation is present in human atherosclerotic lesions, whereas hypermethylation of several genes is implicated in the pathogenesis of atherosclerosis, including extracellular superoxide dismutase, estrogen receptor- α (ER- α), endothelial nitric oxide synthase (eNOS), and 15-lipoxygenase (Turunen et al. 2009). Hiltunen et al. firstly demonstrated that a global genomic hypomethylation with enhanced transcriptional activity was shown during atherogenesis in advanced human, mouse, and rabbit atherosclerotic lesions, further affecting the cellular proliferation and gene expression (Hiltunen et al. 2002). Interestingly, it has been noted that in mice model during early atherosclerosis, local DNA hypermethylation and hypomethylation occurred in normohomocysteinemic mice, and the atherogenic lipoprotein profiles could influence and promote DNA hypermethylation in cultured human macrophages (Lund et al. 2004; Zaina et al. 2005). This suggested that nutritional factors could modify DNA methylation patterns that are likely to be independent of vitamin or homocysteine levels, and

moreover, DNA methylation profiles may serve as early markers of atherosclerosis (Zaina et al. 2005).

Indeed, DNA methylation status could accelerate the process of atherosclerosis through upregulating atherosclerosis-susceptible genes and downregulating atherosclerosis-protective genes (Stenvinkel et al. 2007). The epigenetic changes occur in smooth muscle cells (SMCs), which constitute most of the cellular mass in the arterial wall. For instance, methylation of ER- α promoter was found in SMCs instead of endothelial cells (Post et al. 1999). ER was noted to play the role of active proliferating atheromatous SMCs, and meanwhile, it was the first gene that was well characterized for the association between aging of vascular system and the methylation of CpG islands in normal tissues *in vitro* (Toyota and Issa 1999; Turunen et al. 2009). Moreover, DNA methylation was also investigated to change monocarboxylate transporters (MCTs) by suppressing MCT3 expression, leading to the regulation of SMC proliferation and the development of atherosclerosis (Zhu et al. 2005). Other genes affected by aging are insulin-like growth factor 2 (IGF-2), N33, MYOD1, and cMYC (Toyota and Issa 1999; Hiltunen and Ylä-Herttuala 2003). Furthermore, inducible nitric oxide synthase (iNOS) promoter was heavily methylated in a variety of normal primary endothelial cells and vascular smooth muscle cells, whereas eNOS is hypomethylated in normal endothelium and controlled by cell-specific methylation or histone modifications (Chan et al. 2005; Fish et al. 2005). However, iNOS is expressed in endothelial cells in atherosclerotic lesions which raises the possibility that modifications in epigenetic mechanisms may lead to the changes (Shirodkar and Marsden 2011). Therefore, recent studies showed that aberrant DNA methylation occurs during atherogenesis and contributes to the lesion development and cell proliferation.

2.1.3 DNA Methylation with Cardiovascular Risk and Diseases

DNA methylation was shown to have the correlation with cardiovascular risk and mortality. The association of repetitive element LINE-1 hypomethylation and cardiovascular events, such as ischemic heart diseases and stroke, in cross-sectional and longitudinal analyses of the population has revealed that DNA hypomethylation might predict the diagnosis of CVDs before the clinical onset (Baccarelli et al. 2010). This finding showed that DNA methylation could be an effective marker that predicts the transgenerational cardiovascular risks in early life of adult diseases (Baccarelli et al. 2010). Moreover, DNA methylation was also measured and analyzed in peripheral blood leukocytes from the group of chronic kidney diseases (CKDs). It showed that inflamed patients with CKDs had global hypermethylation in peripheral blood lymphocyte DNA, suggesting the correlation of DNA methylation with increased cardiovascular mortality (Stenvinkel et al. 2007).

With regard to the DNA methylation involved in the heart failure (HF) or cardiomyopathy, there are two interesting studies supporting the essential role of DNA methylation implicated in the pathogenesis of cardiomyopathies. The global methylation profile within the CpG islands of promoters and within gene bodies was

examined between human cardiac tissues from end-stage cardiomyopathic patients who had taken cardiac transplantation and normal controls. This finding showed three angiogenesis-related genes that were differentially methylated, including angiomin-like 2 (AMOLT2), Rho GTPase-activating protein 24 (ARHGAP24), and platelet endothelial cell adhesion molecule (PECAM1) (Movassagh et al. 2010). Of note, hypermethylation in the 5' region of PECAM1 in dilated hearts was correlated with its lower expression, and hypomethylation within the gene body of AMOLT2 was associated with reduced gene expression, whereas hypermethylation within the gene body of ARHGAP24 was correlated with increased gene expression (Movassagh et al. 2010). Therefore, the above study showed that aberrant gene expression implicated in the pathogenesis of HF and a part of the etiologies can be ascribed to the differential DNA methylation. In addition, Haas et al. also reported that an altered methylation pattern is related to dilated cardiomyopathies (DCM) compared with normal controls (Haas et al. 2013). In particular, both lymphocyte antigen 75 (LY75) and adenosine receptors A2A (ADORA2A) showed the significant downregulation in the myocardium of DCM which was mediated by promoter hypermethylation; and meanwhile, in vivo studies of LY75 and ADORA2A in zebra fish demonstrated the function role in adaptive or maladaptive pathways implicated in CVDs (Haas et al. 2013).

Nitric oxide (NO) bioavailability is implicated in the pathogenesis and progression of CVDs (Napoli and Ignarro 2009). Interestingly, it has been shown that the synthesis of NO from the precursor L-arginine could be affected by the complex process of arginine methylation pathways upon the inflammation and oxidative stress (Moncada and Higgs 1993; Rochette et al. 2013). All methylated arginine metabolites could inhibit NO synthesis in an indirect way through competitive blockade of arginine transport in CHF (Closs et al. 1997; Usui et al. 1998). Tang et al. investigated that accumulated plasma levels of methylated arginine metabolites (but not methyl-lysine) were correlated with the presence of left ventricular diastolic dysfunction in chronic systolic heart failure patients (Wilson Tang et al. 2008). Meanwhile, the close association between the methylated arginine metabolites and N-terminal pro-brain natriuretic peptide (NT-proBNP) is also confirmed in this study (Dückelmann et al. 2007; Wilson Tang et al. 2008).

2.2 Histone Modification with CVDs

2.2.1 Histone Modification with Hypoxia and Shear Stress

Hypoxia is majorly responsible for the endothelial phenotype, and the global transcription activity is reported to decline under hypoxic conditions. An ancient eukaryotic response to hypoxia is known as hypoxia-inducible factor-1 (HIF-1) transcription paradigm, and it has also been found that hypoxia induces a global drop in H3K9 acetylation via HDAC upregulation (Gluckman et al. 2008; Wadhwa et al. 2009). Across different cell lines, it has been observed that hypoxic conditions

mediate an increase in the global H3K9 methylation, owing to a rise in G9 histone methyltransferase expression (Waterland and Michels 2007). Moreover, eNOS is well recognized to be of significant importance in regulating the chromatin structure in a cell-specific manner, and it is noteworthy that eNOS proximal promoter histones undergo H3 and H4 acetylation (Waterland and Jirtle 2003).

Vascular endothelium is known to endure a constant shear stress, owing to the physical force of circulation. Shear stress response element (SSRE) is a transcription factor regulating the expression of a few essential genes which are responsible for adapting to the circulatory pressure changes, such as Krüppel-like factor 2 (KLF2), VEGF receptor 2 (VEGFR2), and eNOS (Bogdarina et al. 2007; Heijmans et al. 2008; Tobi et al. 2009). Notably, it has been evidenced that the chromatin-based mechanisms, in particular global acetylation of H3 and H4 and phosphorylation of serine 10 on histone H3 (H3S10), contribute to the transcriptional regulation of these genes (El-Maarri et al. 2007).

2.2.2 Histone Modifications with CAD

As mentioned, the main cause of CADs is atherosclerosis-mediated arterial damage. It is also known that acetylation status is linked to the occurrence of atherosclerosis. HAT modulation is derived from CBP via thrombin signaling through mitogen-activated protein kinase (MAPK) pathway (Reddy and Natarajan 2011). SMCs, serving as the most abundant cell type in the arterial wall, are known to be involved in all the stages of lesion formation in atherosclerosis (Doran et al. 2008). Serum response factor (SRF) and SRF cofactor have the ability to activate CArG elements of SMC gene transcription, the binding of which is associated with methylation and acetylation to histone H3 and H4 residues (Manabe and Owens 2001; Cao et al. 2005; McDonald et al. 2006). In addition, HDAC inhibitors have shown to exacerbate the neointimal lesions in low-density lipoprotein (LDL) receptor-deficient (*Ldlr^{-/-}*) mice, which are known to be atherosclerosis-prone (Choi et al. 2005). Myocardial ischemia and reperfusion injury are also heavily influenced by the HDAC activity, in particular in the case of MI (Granger et al. 2008).

2.2.3 Histone Modifications and HF

HF is regarded as the final stage of all CVDs, and the common causes include ischemic heart disease, hypertrophy, and cardiomyopathy (McMurray and Pfeffer 2005). There are many studies presenting the epigenetic regulation involved in cardiomyocytes. The expression of both class I and class II HDACs has been shown to be associated with cardiac hypertrophy (Kee and Kook 2011). In particular, class I HDACs are commonly considered as injurious to cardiac function (Montgomery et al. 2007; Xie and Hill 2013). It is known that upon the treatment of phenylephrine (PE), the hypertrophic response of cardiomyocytes could be induced with enhanced CBP/p300 transcription activity (Gusterson et al. 2002). Ectopic overexpression of

CBP/p300 is shown to stimulate cardiac growth in decompensated HF (Yanazume et al. 2003). On the global level, trimethylation of histone H3 on lysine-4 (K4TM) or lysine-9 (K9TM) markers has been demonstrated to be linked with the development of HF in a rat model by high-throughput pyrosequencing performed with chromatin immunoprecipitation (ChIP) (Kaneda et al. 2009). Furthermore, the evidence showing the increase of histone acetylation only in the complication-free diabetic subjects in vivo and in vitro may signify that histone acetylation functions as a protective mechanism in the heart (Chen et al. 2009a).

2.2.4 Histone Modifications with Hypertension and Pulmonary Arterial Hypertension (PAH)

Recently, some studies have shown that core histone acetylation is responsible for hypertension and PAH (Mu et al. 2011; Yang et al. 2012; Lee et al. 2013). Environment-induced gene expression alteration in PAH is shown to be caused by certain epigenetic mechanisms (Kim et al. 2011). Therefore, the regulators of acetylation could be used as potential drug targets for treating hypertension and PAH. Valproic acid, a HDAC inhibitor, has shown to prevent the development of hypertension through diminishing transcriptional activity of mineralocorticoid receptor (MR) via increasing its acetylation (Lee et al. 2013). Moreover, HDAC inhibitor-treated spontaneously hypertensive rats (SHR) have successfully decreased vascular inflammation along with blood pressure (Usui et al. 2012). Increased activity of class I HDACs could enhance the pro-inflammatory phenotype of pulmonary adventitial fibroblasts from chronically hypoxic hypertensive calves (PH-Fibs), and therefore, the inhibition of HDACs was able to attenuate PH-Fibs functional activity to induce monocyte migration and adhesion (Li et al. 2011).

2.3 RNA Silencing with CVDs

2.3.1 miRNAs and Cardiac Aging

Aging is a predominant risk factor for CVDs, and the physiological cardiovascular aging is mainly linked with left ventricular hypertrophy, enhanced cardiac fibrosis, diastolic dysfunction, and valvular degeneration (Dai et al. 2012). A variety of miRNAs are found to be differently expressed during the process of cardiac aging with cell-type-specific characteristic (de Lucia et al. 2017). Importantly, miR-21 was the first example of miRNA which was well illustrated to play some role implicated in cardiac function. It has been demonstrated that miR-21 was upregulated selectively in fibroblasts of the failing heart compared with non-failing hearts, activating ERK-MAPK signaling by the inhibition of sprouty homologue 1 (SPRY1) (Thum et al. 2008). Pressure overload of the left ventricle was induced in mice by transverse aortic constriction (TAC), which was followed by the treatment of antagomir-21 or

a control oligonucleotide. Results showed that silencing of miR-21 could reverse the expression of *SPRY1* with the reduction of MAPK activity. Meanwhile, the interstitial fibrosis, cardiac hypertrophy, and dysfunction were repressed 3 weeks after *in vivo* silencing of miR-21 (Thum et al. 2008). Moreover, the myocardial and plasma levels of miR-21 were significantly higher in aortic stenosis (AS) patients compared with normal donors and correlated with echocardiographic mean transvalvular gradients and myocardial collagen expression (Villar et al. 2013). Furthermore, in the investigation of how myocardial ischemia affects miRNA expression, Roy et al. identified that phosphatase and tensin homologue (PTEN) is a direct target of miR-21 in isolated cardiac fibroblasts and miR-21 could modulate the expression of matrix metalloproteinase-2 (MMP-2) through PTEN pathway in the infarct zone (Roy et al. 2009). A decreased expression of PTEN in infarct zone was correlated with enhanced MMP-2 expression (Roy et al. 2009).

miR-34a was another representative miRNA showing an important role in cardiovascular aging (Boon et al. 2013). Boon et al. found that the expression of miR-34a was higher in the heart tissues of C57B16 aging mice compared with young mice. *In vivo* silencing of miR-34a with antisense antagomir Ant-34a in 18-month-old mice decreased the dead cells, and moreover, the age-induced reduction in cardiac function was preserved in miR-34a^{-/-} mice with less cardiomyocyte cellular death and hypertrophy when compared with wild-type controls (Boon et al. 2013). Meanwhile, miR-34a was also assessed in the mouse model of acute myocardial infarction (AMI), and it has been shown that miR-34a was induced after AMI and inhibition of miR-34a by antagomir Ant-34a or LNA-based antimiRs was able to increase cardiac contractile function after AMI (Boon et al. 2013). In addition, phosphatase 1 nuclear targeting subunit (PNUTS), which could repress telomere erosion and DNA damage response, was demonstrated as a novel target gene of miR-34a, further identifying the pro-apoptotic function of miR-34a in cardiomyocytes implicated in the aging process of CVDs (Kim et al. 2009; Landsverk et al. 2010; Boon et al. 2013).

Furthermore, Jazbutyte et al. determined the miRNA expression profiles in neonatal, 4-week, 6-month, and 19-month healthy C57/B16N mice and found that miR-22 was the most prominent upregulated miRNA accompanied by the downregulation of its direct target gene osteoglycin (OGN) during cardiac aging (Jazbutyte et al. 2013). Interestingly, OGN has been shown to be implicated in the regulation of cardiac inflammation, collagen assembly, and cardiomyocyte hypertrophy (Petretto et al. 2008; Van Aelst et al. 2014). Functionally, overexpression of miR-22 was able to accelerate cell senescence and increase the migratory activity of cardiac fibroblasts (Jazbutyte et al. 2013).

2.3.2 miRNAs and HF

Several studies have reported the potential of circulating miRNAs for the diagnosis of HF. The miRNA signature of failing myocardium is comprised of the dysregulated miRNAs such as miR-1-1, miR-195, miR-199a-1, miR-199b, and miR-221

(Akat et al. 2014). miR-1 family has been investigated as the muscle-specific miRNA and one of the essential regulating miRNAs implicated in cardiogenesis and skeletal muscle development (Lagos-Quintana et al. 2002; Zhao et al. 2007). miR-1 is highly expressed in the heart muscles, and deletion of miR-1-2 could lead to the cardiac defects of left ventricular malfunction progressing to a DCM (Zhao et al. 2007; Rao et al. 2009). Moreover, miR-1 was shown to downregulate in the early stage of cardiac hypertrophy in the hearts of C57BL/6 mice with TAC (Sayed et al. 2007). Several important regulatory genes are demonstrated as the target genes of miR-1, such as insulin-like growth factor-1 (IGF-1), twinfilin-1, a cytoskeleton regulatory protein, and heart-type fatty acid-binding protein-3 (FABP3), which are implicated in the process of cell growth, cellular differentiation or cardiac cytoskeleton, and metabolism (Elia et al. 2009; Li et al. 2010; Varrone et al. 2013).

In acute heart failure (AHF), a subset of 7 circulating miRNAs (miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-199a-3p, and miR-652-3p) were validated to differ mostly in healthy donor controls and AHF patients (Ovchinnikova et al. 2016). In particular, AHF patients were shown to get further downregulation of the above circulating miRNAs within 48 h after admission to hospital (Ovchinnikova et al. 2016). Voellenkle et al. also investigated the miRNA expression profile in peripheral blood derived from a population of CHF patients affected by ischemic and nonischemic DCMs (Voellenkle et al. 2010). Three circulating miRNAs, including miR-107, miR-139, and miR-142-5p, were found to significantly downregulate in all HF patients when compared with healthy control donors (Voellenkle et al. 2010). Interestingly, miR-125b and miR-497 were only shown to decrease in ischemic patients, whereas miR-142-3p and miR-29b were significantly enhanced in nonischemic patients (Voellenkle et al. 2010).

2.3.3 miRNAs as Potential Biomarkers

miRNAs can be easily detected by real-time polymerase chain reaction (qRT-PCR) or microarrays in body fluid, including blood, saliva, or urine, which provides the opportunities to use these small molecules as biomarkers for human diseases (Mitchell et al. 2008; Kroh et al. 2010; Gallo et al. 2012; Tijssen et al. 2012). Importantly, cell-free blood serum and plasma miRNAs are the most stable and suitable sources for clinical application such as the diagnosis of disease given that the process of sample collection is simple and reproducible as well as the expression of miRNAs in the blood is stable even in the long term (Chim et al. 2008; Mitchell et al. 2008). Interestingly, it has been found that genetic exchange of mRNA and extracellular miRNAs between cells could be accomplished through microvesicles (MVs) or exosomes (Hunter et al. 2008). These microvesicles and exosomes containing miRNAs could facilitate the communication between cells or organs in the body. Exosomes are a population of extracellular vesicles (50–100 nm in diameter). They are released from cells in the process of inward budding of endosomal membrane that are fused with plasma membrane. Compared with exosomes, MVs

are larger-sized vesicles (50–1000 nm in diameter) that are released from cells via outward budding (Raposo and Stoorvogel 2013; Lawson et al. 2016).

In CVDs, it has been noted that exosomes are manipulated by cardiovascular cells for intercellular communication as paracrine signaling mediators. Normally, the passenger strands of miRNAs, called star miRNAs (miRNA*), are considered to have no important biological function since they undergo intracellular degradation (Bartel 2004). Surprisingly, cardiac fibroblasts could secrete exosomes that are enriched with several miRNA passenger strands, in particular miR-21-3p, which has been identified to take the essential role to induce the cardiomyocyte hypertrophy (Bang et al. 2014). Moreover, the inhibition against miR-21-3p in mice with Ang II-induced cardiac hypertrophy could also reduce the pathological development of cardiac hypertrophy. Accordingly, it showed that cardiac fibroblast-derived miR-21-3p could serve as an important paracrine signaling effector in the process of fibroblast-derived cardiomyocyte hypertrophy, suggesting the potential therapeutic potential in HF patients in the future (Bang et al. 2014). Furthermore, Hergenreider et al. reported that endothelial-derived miR-143/145-containing vesicles could induce an atheroprotective SMC phenotype (Hergenreider et al. 2012). The shear-responsive transcription factor KLF2, which is a crucial mediator of endothelial gene expression patterns induced by atheroprotective flow, could directly bind to the promoter of miR-143/145 cluster and induce its upregulation (Dekker et al. 2006). In addition, extracellular vesicles generated from KLF2-transduced mouse endothelial cells could protect the mice from atherosclerotic lesion formation in the aorta of ApoE^{-/-} mice, further demonstrating the protective role of KLF2 in miR-143/145-dependent manner (Hergenreider et al. 2012). Altogether, the atheroprotective stimuli could initiate the cross talk between endothelial cells and SMCs by the miRNAs-extracellular vesicle mechanisms, implying the promising therapy for atherosclerosis.

3 The Environment, Epigenetics, and CVD Risk

Today, approximately 7.6 billion people around the world from diverse natural environments live in their own lifestyle in different societies. Of note, lifestyle factors, society, and natural environment not only influence on daily living but also on the genome function through the dynamic and plastic epigenetic mechanisms including DNA methylation, histone modification, or miRNA aberrant expression (Alegria-Torres et al. 2011). As the life expectancy increases, the prevalence of chronic diseases will increase. In particular, it is estimated that by 2030, 40.5% of the population in the USA may have some forms of CVDs (Heidenreich et al. 2011). Therefore, the better understanding and early identification of how the natural, social, and personal environments modify the outcome of CVDs are highlighted for our prevention and management.

3.1 *Natural Environmental Exposures*

In environmental studies, it is noted that air pollution, circadian rhythm, and sunlight exposure are important factors that proved to be linked with CVDs that are partly regulated by epigenetic modifications. Firstly, exposure to air pollutants has been associated with the increased risk of CVDs (Du et al. 2016). Evidence showed that exposure level to fine metal-rich particulates (particulate matter <2.5 μm in diameter [PM_{2.5}]) was demonstrated to be inversely correlated with the methylation of mitochondrial DNA (mtDNA) in the blood and participants with higher level of mtDNA methylation are more susceptible to heart rate variability due to the effect of PM_{2.5} exposure (Byun et al. 2016). Moreover, it was also shown that older participants with higher toll-like receptor 2 (TLR2) methylation were more prone to adverse autonomic effect of PM_{2.5} exposure (Zhong et al. 2015). Dietary modulation by flavonoids might reduce the above effect through the attenuation of TLR2 methylation. In addition, cardiovascular events, such as MI, sudden cardiac death, and pulmonary embolism, are recognized to have circadian rhythmicity with the high incidence in the morning, suggesting that chronic circadian disruption may enhance the susceptibility to CVDs (Oishi 2009). The evidence demonstrated that CLOCK, a transcription factor for circadian function, has the intrinsic HAT activity, whereas SIRT1 was shown to function as a HDAC that counteracts CLOCK activity to manage circadian chromatin remodeling (Bellet and Sassone-Corsi 2010). It has been identified that vitamin D deficiency has contributed to cardiovascular events such as MI, stroke, and HF (Nemerovski et al. 2009). More importantly, adequate vitamin D supplementation or sunlight exposure are beneficial for the prevention of CVDs (Wimalawansa 2016). Interestingly, vitamin D could be interacted with epigenome on multiple levels, including the methylation alteration of several critical genes such as vitamin D receptor (VDR) and the enzyme 25-hydroxylase (CYP2R1), as well as the histone modification of VDR proteins through HATs or HDACs (Fetahu et al. 2014).

3.2 *Personal Environment*

Smoking, diet, alcohol consumption, and sleeping habits are important factors that may have impacts on CVDs. Prenatal exposure to tobacco smoke increases the risk of diseases later in child's life which is partly mediated by the hypomethylation of AluYb8 and hypermethylation of AXL and PTPRO in exposed children compared with controls (Breton et al. 2009). It suggested that lifelong effect of in utero exposures could be mediated by aberrant DNA methylation. Jiang et al. also found that persistent nicotine exposure selectively inhibited the expression of TBX5 and GATA4 by promoter hypermethylation, leading to cardiac function impairment with repressed left ventricular ejection fraction (Jiang et al. 2017). On the other hand, diet nutrition is another crucial factor influencing chronic disease susceptibility by

the epigenetic mechanism. It is supported by the evidence that maternal diet habit during pregnancy could modify the DNA methylation pattern of specific genes, resulting in some phenotypic changes such as blood pressure, body weight, and coat color (Waterland and Jirtle 2003; Bogdarina et al. 2007). Calorie restriction (CR) is proved to be the most effective nutritional intervention to increase longevity and prevent chronic diseases such as CVDs, hypertension, and diabetes (Omodei and Fontana 2011). DNA methylation drift with age was demonstrated to be delayed by CR in mice and monkeys, showing that epigenetic drift is a good biomarker of life span (Maegawa et al. 2017). In addition, sleeping deprivation also increases the risk of CVDs, which may be mediated by the enhanced global methylation status that leads to systemic cell injury and uncompensated oxidative stress (Everson et al. 2005; Trivedi et al. 2017).

4 Technical Overview of Platform Used in Epigenetic Study

4.1 *DNA Methylation with Regional and Genome-Wide Approach*

In the genome, usually a proportion of potential target sequences could be methylated so that the distribution of methyl marks can carry and express epigenetic information through transcriptional silencing (Laird 2010). Both the genome-wide scale and regional DNA methylation profiles have critical implications for better understanding of how the specific loci of genome express under some condition and how the epigenetic aberration contributes to the pathogenesis of diseases (Robertson 2005). Recent advances in examination and quantification of DNA modifications facilitate the epigenetic changes to be connected to phenotypic consequences on an unprecedented scale (Plongthongkum et al. 2014).

With regard to the sequence-specific DNA methylation detection and analysis, methylation-dependent pre-treatment of DNA should be performed at first to reveal the presence or absence of methyl group at cytosine residues before DNA amplification or hybridization (Fraga and Esteller 2002; Laird 2003). There are three kinds of methods for methylation-dependent pre-treatments including bisulfite conversion, endonuclease digestion, and affinity enrichment (Laird 2010).

4.1.1 Bisulfite Conversion

The utility of sodium bisulfite on denatured genomic DNA is based on the function of bisulfite to selectively deaminate the unmethylated cytosine residue, providing the basis to distinguish the methylated and unmethylated CpG sites (Shapiro et al. 1970; Hayatsu 2008). The bisulfite treatment of DNA could deaminate unmethylated cytosine to uracil, leaving the methylated cytosine unchanged.

Therefore, bisulfite conversion enables the application of new techniques to detect the methylation status at a particular region (Dahl and Guldberg 2003; Laird 2003; Dupont et al. 2004). Bisulfite-treated genomic DNA was at first analyzed and demonstrated by Sanger sequencing of cloned polymerase chain reaction (PCR) amplification at the single base pair level (Frommer et al. 1992; Paul and Clark 1996). Methylation-specific PCR (MSP) can detect the DNA methylation status of specific genes with high sensitivity and specificity (Herman et al. 1996).

4.1.2 Endonuclease Digestion

Restriction enzyme was found in bacteria and archaea by working as a defense mechanism against invading viruses, which was now used as a powerful tool in molecular biology (Roberts et al. 2003). Each sequence-specific restriction enzyme was accompanied by DNMTs that could protect DNA from restriction defense system through the methylation of recognition site (Laird 2010). Predicted modifications in restriction enzyme sites following bisulfite reaction and amplification of the product by the PCR were used to assess the methylation of CpG sites (Sadri and Hornsby 1996). *HpaII* and *SmaI* are the most widely used methylation-sensitive restriction enzymes for DNA methylation studies, which are not repressed by CpG methylation (Singer-Sam et al. 1990; Laird 2010).

4.1.3 Affinity Enrichment

The most recent way to enrich genomic methylated DNA is to do affinity purification and enrichment. One approach is to use MBDs with affinity for methylated CpG sites; alternatively, another way is to use monoclonal antibodies that are specific for 5-methylcytosine (5^{me}C , in the context of denatured DNA) to immunoprecipitate methylated DNA (Cross et al. 1994; Selker et al. 2003; Weber et al. 2005). Of note, the affinity of methylated DNA was firstly identified with the rat chromosomal protein MeCP2 (Cross et al. 1994). Meanwhile, the immunoprecipitation approach, which is followed by hybridization to a tiling array or microarray, is regarded as MeDIP or mDIP (Weber et al. 2005; Keshet et al. 2006; Weber et al. 2007). Affinity-based approaches are to measure the density of methylation in a selected region (Zilberman and Henikoff 2007). Thus, the target regions with sparse methylation sites might be difficult to differentiate the methylated and unmethylated sites, especially for mammalian genomes (Weber et al. 2007; Zilberman and Henikoff 2007).

4.1.4 Genome-Wide Approaches for Methylation

Over the past years, a large amount of approaches for genome-wide methylation analysis have been reported. As a type of two-dimensional electrophoresis, restriction landmark genomic scanning (RLGS) was the first technique in the genome-wide assessment of both genomic and epigenetic modifications (Fischer and Lerman 1979; Rush and Plass 2002). RLGS uses methylation-sensitive restriction enzymes to create the “landmarks”, and therefore, methylation difference could be detected by the presence of radiolabeled locus in unmethylated profile. In methylated profile, no radiolabeled RLGS fragment is identified because the methylation-sensitive enzyme fails to digest the DNA (Hatada et al. 1991; Rush and Plass 2002). RLGS analysis of different tumor samples has showed the relevant cancer-specific patterns of methylation, contributing to our understanding of the role of methylation implicated in carcinogenesis (Costello et al. 2000; Allegrucci et al. 2007). Nowadays with the advent of next-generation sequencing (NGS) techniques, the use of RLGS is replaced by the sequencing approaches. Sequence-based technique is more flexible and high-throughput to show the methylation patterns on a genome-wide scale within specific sequence since it can read allele-specific DNA methylation after restriction enzyme enrichment (Cokus et al. 2008; Laird 2010; Davey et al. 2011). However, it is restricted to the sequence library biases (Laird 2010).

4.2 Approaches for Histone Modification Analysis

Chromatin state could directly regulate transcription, and the cross talk between chromatin and transcription is dynamic in cellular process (Berger 2007). Importantly, histone modifications are crucial epigenetic mechanisms for eukaryotic gene regulation. ChIP assay serves as the primary powerful technique to investigate the defined genomic locations associated with histone modifications within cells or tissues (Spencer et al. 2003). In standard ChIP protocol, chromatin is released from lysed cells, digested into fragments and then selectively bound with specific antibodies against the protein of interest (Geng et al. 2011). The chromatin-antibody complex is removed, and then ChIP DNA could be further purified and analyzed by a series of approaches, such as conventional PCR, quantitative PCR, hybridization to array (ChIP-chip), or sequencing (Milne et al. 2009). Standard ChIP procedure is subjected to the multiple steps, the size of cell population, as well as the inefficient immunoprecipitation. The introduction of ChIP-chip enables the genome-wide profile of DNA-protein interaction (Ren et al. 2000). During recent years, ChIP coupled with high-throughput sequencing (ChIP-Seq) is increasingly used to map the high-resolution genome-scale views of protein-DNA interactions including transcription factor bindings, histone modification, DNA methylation, and nucleosome positioning (Park 2009; O’Geen et al. 2011). With wider coverage, less noise, single base pair resolution, and deeper sequencing, ChIP-Seq provides several advantages when compared to array-based ChIP-chip (Zhang et al. 2008;

Park 2009). In 2007, the first comprehensive genome-wide map produced by ChIP-Seq showed 20 histone methylation markers, histone variant H2A.Z, RNA polymerase II, and CTCF (Barski et al. 2007; Mikkelsen et al. 2007). As the cost of sequencing decreases and institutional support grows, ChIP-Seq is likely to work as the main approach for exploring histone modifications in the future (Park 2009).

4.3 Approaches for miRNA Studies

Over the past years, miRNAs have been extensively studied in cellular processes, development, human diseases, and in particular, human cancers (Bartel 2009). It was reported that approximately 2600 miRNAs regulate more than 60% of human genomes involved in the regulation of cellular growth and death (Friedman et al. 2009; Shu et al. 2017). Therefore, a number of measurement platforms have been developed to examine the relative miRNA expression in different biological samples for better understanding of miRNA roles implicated in human diseases (Shingara et al. 2005; Chen et al. 2008; Willenbrock et al. 2009; Mestdagh et al. 2014). For miRNA studies, three main techniques are included including qRT-PCR, hybridization (DNA microarrays), and small RNA sequencing (Kong et al. 2009). The choice of platform depends on the specific experimental goals. It has been found that TaqMan qPCRarray might show superior sensitivity and specificity when compared with microarray (Chen et al. 2009b). However, microarray and qPCR platforms are limited to the number of probes or assays available, which in turn is subjected to the update of miRBase (Chen et al. 2009b; Mestdagh et al. 2014). In addition, by comparison of microarray (Exiqon's LNA-based microarrays) and NGS (Illumina's GA-II sequencing platform), microarray expression analysis might offer both high specificity and sensitivity, while NGS appears the ability to discover new sequence variants (Willenbrock et al. 2009).

5 The Application of Epigenetic Therapy to Human Diseases

Given that the epigenetic etiology may contribute to the pathogenesis of human diseases, this encourages the development of new promising "epigenetic therapy." To date, several agents have been discovered that could modify the epigenetic information in a manner that is not specific to genes, including inhibitors of DNMTs and HDACs (Egger et al. 2004; Miyamoto and Ushijima 2005). Some of these agents are tested in clinical trials, while gene-specific approaches are under exploration (Lubbert 2000; Issa et al. 2004; Wagner et al. 2010).

5.1 *Demethylation by 5-Azacytidine, Decitabine, and Zebularine*

Since DNA methylation can be reversible silencing, the expression and function of TSGs, miRNAs, or lncRNAs that have undergone inactivation by DNA methylation will be restored by state-of-the-art epigenetic therapy (Wang and Chim 2015). DNMTs, especially DNMT1, are known to maintain CpG methylation (Robert et al. 2003). The DNMT1 inhibitor, 5-aza-2'-deoxycytidine (5-aza-dC, decitabine), forms covalent attachment with DNMT1, resulting in the degradation of DNMT1 and therefore DNA hypomethylation (Šorm et al. 1964; Christman 2002). Importantly, pharmacological grade 5-aza-dC has been identified to increase response rate and survival in patients with myelodysplastic syndrome (Silverman et al. 2002; Fenaux et al. 2009). Compared with 5-aza-dC used in high dose of 75–100 mg/m²/day, low-dose treatment of 5–20 mg/m²/day for chronic myelogenous leukemia patients in phase I study appeared more effective (Kantarjian et al. 2003; Issa et al. 2004). The major adverse effect of 5-aza-dC was myelosuppression and toxic, and the administration of 5-aza-dC is restricted to the short half-life and cannot be given orally (Issa et al. 2004). To further improve the stability and efficacy of 5-aza-dC, some cytidine analogs are under development (Yang et al. 2010). For instance, zebularine is a chemically stable cytidine analog, which could inhibit DNMTs and be administered orally or intraperitoneally (Cheng et al. 2003; Yoo et al. 2008). Moreover, zebularine showed the preference of depleting DNMT1 and induction of cancer-related antigens in cancer cells rather than normal fibroblasts (Cheng et al. 2004). However, the drawback is that higher concentration of zebularine is needed to get similar effect of demethylation in cells when compared with 5-aza-dC (Cheng et al. 2004).

5.2 *Demethylation by Non-nucleoside Analogs*

Different from cytidine analogs, non-nucleoside DNMT inhibitors, such as hydralazine, procaine, and procainamide, have limited activity of demethylation and less cytotoxicity as they are not incorporated into DNA (Chuang et al. 2005). For instance, the vasodilator agent hydralazine was shown to induce demethylating activity in human breast adenocarcinoma cells (Segura-Pacheco et al. 2006). Moreover, the inhibitory function of hydralazine with DNMTs was rationalized through the interaction of similar pharmacophore as established by deoxycytidine (Singh et al. 2009). Another approach to inhibit DNMT1 is to use the short-chain oligodeoxynucleotides. MG98 is a 20 base pair antisense oligodeoxynucleotide, which specifically binds to the 3' UTR of human DNMT1 mRNA (Amato 2007). Preclinical data showed that a patient with renal cell carcinoma treated at 80 mg/m² of MG98 showed partial response (Stewart et al. 2003). A number of phase I/II clinical trials are investigating the safety and efficacy of MG98 in several solid and hematopoietic cancers (Amato 2007).

5.3 HDAC and HAT Inhibition

Histones can be acetylated and deacetylated, and two opposing classes of enzymes are involved in determining the pattern of histone acetylation, HDACs and HATs. Several leukemogenic transcription factors depress the expression of specific genes through aberrant recruitment of HDAC, and consequently, HDAC-dependent abnormal transcription repression contributes to the oncogenic mechanism of acute promyelocytic leukemia and non-Hodgkin's lymphoma (Dhordain et al. 1998; Lin et al. 1998). Acetylation results in the relaxation of chromatin structure and transcriptional activation of many genes, which might serve as a powerful mediator of tumor response to chemotherapy or adaptation environment to changes such as hypoxia (Grunstein 1997; Cao et al. 2011).

5.3.1 HDAC Inhibitors

Importantly, a number of HDAC inhibitors are found to induce cellular growth, differentiation, and/or apoptosis in transformed cells in culture or in tumors (Yoshida et al. 1990; Kijima et al. 1993; Kim et al. 1999). In particular, the cell cycle kinase inhibitor, p21^{WAF1}, can be induced with the specific HDAC inhibitor trichostatin A (TSA) in p53-independent manner in NIH3T3 cells, which provides new insights to cancer therapy that could arrest tumor cells in p53-independent way (Xiao et al. 1999). Of note, as autophagy is found to be involved in the pathological cardiac remodeling, TSA treatment has been shown to reduce both load- and agonist-induced hypertrophic growth and depress the autophagy in Beclin 1 transgenic mice, suggesting the promising strategy of the HDAC inhibitor targeting autophagy (Cao et al. 2011).

To date, the HDAC inhibitors, vorinostat and romidepsin, have been used for the treatment of refractory cutaneous T cell lymphoma (CTCL) at clinic (Duvic et al. 2007; Piekarz et al. 2009). Approved by FDA for clinical application, either of them was demonstrated to have the single-agent clinical activity with significant and sustainable responses in CTCL patients, making an important therapeutic option for CTCL treatment (Olsen et al. 2007; Whittaker et al. 2010). At the same time, some newer compounds such as panobinostat, givinostat, and mocetinostat are currently being clinically assessed (Qiu et al. 2013; West and Johnstone 2014).

5.3.2 HAT Inhibitors

Different subtypes of HATs enable to catalyze the histone acetylation on specific lysine residues (Dekker and Haisma 2009). HAT families have been extensively studied including the GNAT family, the p300/CBP family, the MYST family, and the Rtt109 family (Hodawadekar and Marmorstein 2007; Nagy and Tora 2007). HATs are identified to implicate in several crucial cell proliferation pathways, and

therefore disruption of these enzymes leading to chromosomal translocations might be involved in the pathogenesis of leukemia (Sobulo et al. 1997; Perez-Campo et al. 2009; Chen et al. 2010). Therefore, the above findings make HAT an attractive target for cancer therapy. The first HAT inhibitor to be described for selectively targeting the HAT p300/CBP family was bisubstrate analog inhibitor (Lau et al. 2000). Spermidinyl-CoA-based HAT inhibitors are found to block DNA repair and serve as cancer-specific and radiosensitive drug (Bandyopadhyay et al. 2009). A novel inhibitor of p300 HAT activity, C646, is a relatively potent and cell-permeable small molecule, showing the ability to slow the cancer cell growth and impede intracellular histone acetylation (Bowers et al. 2010). Of note, in heart diseases, the balance of histone acetylation and deacetylation plays the role of regulating hypertrophic response of cardiomyocytes to phenylephrine (Gusterson et al. 2003). Inhibition of transcription coactivator p300 or CBP HAT reduced the cardiac hypertrophy, whereas activating them promoted hypertrophy in HAT-dependent manner (Gusterson et al. 2003).

5.4 Combined Therapies with Demethylating Drug and HDAC Inhibitors

Epigenetic drug, no matter demethylating drug or HDAC inhibitor, results in the reexpression and reactivation of tumor-suppressive genes, miRNAs, and lncRNAs, which play important roles involved in the cellular process and function (Wang and Chim 2015). Interestingly, these two inhibitors can be used together to improve its effectiveness. It has been firstly demonstrated that sequential exposure to 5-azacytidine and butyrate (HDAC inhibitor) had a strong synergistic effect to induce the β -adrenergic receptors in HeLa cells (Jahangeer et al. 1982). Moreover, the combination of 5-azacytidine and TSA (HDAC inhibitor) treatment on esophageal squamous cell carcinoma could lead to the functional reactivation of several TSGs (Yamashita et al. 2002). Moreover, DNMT1 contributed to the development of lung cancer in murine model, and importantly, simultaneous 5-azacytidine (DNMT inhibitor) and phenyl butyrate (HDAC inhibitor) treatment led to the re-expression of TSGs, indicating that the combined epigenetic therapy could be effective for preventing murine lung cancer (Belinsky et al. 2003). In addition, clinical trials are developed targeting two epigenetic modifications in cancer patients. It has shown that combined epigenetic therapy with low-dose 5-azacytidine and entinostat (HDAC inhibitor) led to the durable response in patients with recurrent metastatic non-small cell lung cancer (Jurgens et al. 2011). In addition, epigenetic therapy can be used for sensitive drug-resistant tumors followed by chemotherapy or immunotherapy, showing a good response (Weber et al. 1994; Plumb et al. 2000).

Despite the advantages of epigenetic therapy, the therapeutic use is still very preliminary, and there are some concerns about the clinical application. One concern is regarding the nonspecific effect to normal cells, in which DNA methylation might enforce the inactivation of genes in normal cells or activation of oncogenes,

leading to the potential mutagenicity (Jackson-Grusby et al. 1997; Liang et al. 2002; Sato et al. 2003; Egger et al. 2004). Moreover, both HDAC and methylation inhibitors are cytotoxic, which could induce the cellular apoptosis and/or cell cycle arrest by activating p21 or p53 (Karpf et al. 2001; Marks et al. 2003). Therefore, the clinical application of epigenetic therapy needs to be examined in patients to get a better understanding and response.

6 Future and Prospective

Epigenetic modifications have shown to contribute to several human diseases, including CVDs. Of them, DNA methylation is major epigenetic modification, which is more extensively studied compared with histone modification and miRNA alternations. In particular, DNA methylation of specific genes or miRNAs, as well as histone modifications and aberrant miRNA expression, have been noted in different subtypes of CVDs, suggesting the critical role of epigenetics involved in the pathogenesis of CVD. Interestingly, some environmental exposures could change the epigenetic markers, and therefore, it is promising to find epigenetic therapies to such reversible modifications. Some environmental factors including air pollution, smoking, aging, circadian rhythm, and diet nutrition, are found to be associated with epigenetic alternations in CVD patients when compared with those in normal controls (Dong et al. 2002; Oka et al. 2009; Baccarelli and Ghosh 2012). Recent studies have discovered how epigenetic mechanisms are involved in the pathogenesis of CVDs. As a result, there has been a rapid increase in epigenetic therapies for CVDs, such as the demethylating drug, HDAC inhibitors, or miRNA mimics/inhibitors. However, the difficulties of clinical application for CVDs cannot be underestimated. The dosage and adverse effect of drug, route of administration, and drug interactions, are crucial factors to be considered to avoid the toxicity. It is apparent that we are still at the beginning of understanding the substantial contribution of epigenetics to CVDs, and elucidating the complete epigenetic information map will be an exciting challenge for us and eventually result in a clearer direction to illustrating the diseases as well as the novel therapeutic concepts.

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