FORUM REVIEW ARTICLE

The Role of Redox Signaling in Epigenetics and Cardiovascular Disease

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Abstract

Significance: The term epigenetics refers to the changes in the phenotype and gene expression that occur without alterations in the DNA sequence. There is a rapidly growing body of evidence that epigenetic modifications are involved in the pathological mechanisms of many cardiovascular diseases (CVDs), which intersect with many of the pathways involved in oxidative stress. **Recent Advances:** Most studies relating epigenetics and human pathologies have focused on cancer. There has been a limited study of epigenetic mechanisms in CVDs. Although CVDs have multiple established genetic and environmental risk factors, these explain only a portion of the total CVD risk. The epigenetic perspective is beginning to shed new light on how the environment influences gene expression and disease susceptibility in CVDs. Known epigenetic changes contributing to CVD include hypomethylation in proliferating vascular smooth muscle cells in atherosclerosis, changes in estrogen receptor- α (ER- α) and ER- β methylation in vascular disease, decreased superoxide dismutase 2 expression in pulmonary hypertension (PH), as well as trimethylation of histones H3K4 and H3K9 in congestive heart failure. **Critical Issues:** In this review, we discuss the epigenetic modifications in CVDs, including atherosclerosis, congestive heart failure, hypertension, and PH, with a focus on altered redox signaling. **Future Directions:** As advances in both the methodology and technology accelerate the study of epigenetic modifications, the critical role they play in CVD is beginning to emerge. A fundamental question in the field of epigenetics is to understand the biochemical mechanisms underlying reactive oxygen species-dependent regulation of epigenetic modification. *Antioxid. Redox Signal.* 18, 1920–1936.

Introduction

EPIGENETICS REFERS TO THE CHANGES in the phenotype mediated by altered gene expression, which are not the result of alterations in the DNA sequence. Epigenetic mechanisms can be acquired and/or heritable and constitute a means by which gene–environment interactions occur (10). There are three major mechanisms of epigenetic regulation: (i) methylation of CpG islands, mediated by DNA methyltransferases; (ii) modification of histone proteins; and (iii) microRNAs (miRNAs) (Fig. 1). These modifications result in a variable expression of identical genetic information based on the surrounding conditions and lead to enhanced expression or silencing of genes. There is a substantial interaction between these epigenetic mechanisms. Even though epigenetic variability of genetic information is a part of normal development

Innovation

Epigenetic modifications are becoming increasingly recognized as playing a causative role in cardiovascular disorders (CVDs). In some instances, when evaluated, these epigenetic changes mediate their effect through alterations in redox signaling. For example, H₂O₂ recruits DNMTs to chromatin and can cause hypomethylation in atherosclerosis. Also, epigenetic silencing of SOD2 decreases production of H₂O₂ and promotes SMC proliferation. As more epigenetic changes are identified in CVDs, understanding the impact on redox signaling and the contributions of oxidative stress to the disease process will be important and, based on the epigenetic literature to date, will also be high yield.

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FIG. 1. Schematic of epigenetic mechanisms. Histone modifications, DNA methylation, and RNA-mediated gene silencing constitute three distinct mechanisms of epigenetic regulation. Histone (chromatin) modifications refer to covalent post-translational modifications of N-terminal tails of four core histones (H3, H4, H2A, and H2B). DNA methylation is a covalent modification of the cytosine (C) that is located 5' to a guanine (G) in a cytosine-paired-with-guanine (CpG) dinucleotide. MicroRNAs (miRNA) inhibit translation or decrease mRNA stability by binding to specific sites usually in the 3'-untranslated region (3'UTR) of target messages. RNA-induced silencing complex, or RISC, is a multiprotein complex that incorporates one strand of miRNA.

and differentiation, it also underlies exogenous stimuli, for example, smoking or drug abuse, and as such may reflect the role of these factors on the development of diseases (37). There is a large body of evidence that epigenetic modifications are involved in the pathological mechanisms of many diseases, including cancer (46) and several human syndromic disorders, such as Prader-Willi, Angelman, Silver-Russell, and Beckwith-Wiedemann syndromes (9,47,77).

In health, there is a balance between reactive oxygen species (ROS) generation and the activity of enzymatic and nonenzymatic antioxidant systems that scavenge or reduce ROS concentrations (49). Redox imbalance caused by increased ROS production and/or reduced antioxidant reserve causes oxidative stress, a disruption of redox signaling and control (63). The tightly regulated production of ROS can modulate the activity of diverse intracellular molecules and signaling pathways (a mechanism commonly termed redox signaling), with the potential to induce highly specific acute and chronic changes in the cell phenotype. Redox signaling plays a pivotal role in many disorders, for example, vascular smooth muscle proliferation, atherosclerosis, angiogenesis, cardiac hypertrophy, and fibrosis (39).

Elevated levels of ROS arising from alterations in cellular metabolism and inflammatory responses constitute a key risk state for DNA damage. DNA repair requires dynamic changes in surrounding chromatin, including changes in nucleosome positioning and histone modifications (118,142). Histone acetylases and deacetylases (HDACs) localize to the sites of DNA damage to facilitate repair by increasing the access of repair proteins to the break site, repressing transcription at the sites of damage, restoring the local chromatin environment after repair is complete, and turning off the DNA damage response.

Previously published comprehensive reviews on epigenetic changes in cardiovascular disease (CVD) have focused on certain disease processes (69,139), the underlying mechanisms (6,51,122), and introduced novel concepts such as epigenomics (101). In this review, we will focus on the interaction between epigenetic changes and oxidative stress. A fundamental question in the field of epigenetics is to understand the biochemical mechanisms underlying ROS-dependent regulation of epigenetic modification, which may open the door to identifying new therapeutic targets (32). This review provides an introduction to epigenetic mechanisms such as DNA methylation, histone modifications, and RNA-based modifications and how these changes pertain to the development of CVD (Fig. 2). Although the literature remains sparse, the interactions between redox signaling and epigenetics will be highlighted.

DNA Methylation

DNA methylation refers to the covalent attachment of a methyl group to the C5 position of cytosine residues in the cytosine-paired-with-guanine (CpG) dinucleotide sequences. Changes in the methylation status in the promoter region in the areas enriched in the CpG dinucleotides (CpG islands) have been intensively studied (57). It has been found that hypermethylation of the CpG islands is associated with epigenetic silencing (64). DNA methylation is involved in normal cellular control of gene expression and is dynamically regulated (11). The recent discovery of active DNA demethylation in postmitotic cells and dynamic DNA methylation and demethylation *in vivo* challenges the conventional view that DNA methylation is a stable epigenetic mark (72,73). Dynamic changes in DNA methylation may therefore link the

FIG. 2. Cardiovascular epigenetics. Epigenetic changes mediate disease in various aspects of cardiovascular pathology. In this figure, the anatomical targets of epigenetic injury are aligned with the genetic changes observed.

FIG. 3. Schematic of DNA methylation. (A) DNA methylation refers to the covalent attachment of a methyl group to the C5 position of cytosine residues in CpG dinucleotide sequences called CpG islands. Hypermethylation of CpG islands represented by the red line is associated with epigenetic silencing and ultimately minimal expression of the relevant gene. (B) The presence of the methyl group (Me) at a specific CpG dinucleotide site (C-G) may directly block DNA recognition. (C) Alternatively, the presence of the methyl group may block binding by some transcription factors (TFs). Proteins may preferentially bind to methylated DNA (BP), thereby blocking TF access to these regulatory elements.

environment to disease pathogenesis (59). DNA methylation states may also vary over an individual's lifetime, and only recently have these alterations been studied in the realm of CVDs, such as atherosclerosis, inflammation, hypertension, and diabetes (42,80).

CpG methylation can suppress transcription by several mechanisms (Fig. 3). The presence of the methyl group at a specific CpG dinucleotide site may directly block DNA recognition and binding by some transcription factors (4). In other instances, some proteins may preferentially bind to methylated DNA, thereby blocking the transcription factor access to these regulatory elements (51). In atherosclerosis, the binding of certain transcription factors, such as hypoxia-inducible factor-1 α (HIF-1 α) (136), myc (104), and insulator CTCF (CCCTC-binding factor), has been shown to be affected by DNA methylation *via* this mechanism. A family of methyl-CpG-binding proteins has recently been recognized that specifically bind to methylated CpGs, thereby contributing to transcriptional repression by recruiting histone-modifying proteins. These include the MBD protein family (MBD1, MBD2, MBD4, and MeCP2), Kaiso and Kaiso-like proteins, and SRA domain proteins (*e.g.*, SUVH9 and SUVH2) (110). These methyl-CpG-binding proteins can directly repress transcription, prevent the binding of activating transcription factors, or recruit enzymes that catalyze histone post-translational modifications and chromatin-remodeling complexes that alter the structure of chromatin and actively promote transcriptional repression (91).

A family of DNA methyltransferase enzymes (DNMTs) is involved in *de novo* DNA methylation and maintaining methylation. DNMTs facilitate the epigenetic control of gene expression by cytosine methylation. The regulation of the expression of DNMTs represents an additional mechanism of epigenetic control (48) and is mediated transcriptionally or by post-translational means. DNMTs recognize CpGs within double-stranded DNA as substrates. DNMT1 maintains DNA methylation and occurs in step with DNA replication. Thus,

FIG. 4. Schematic of DNA methyltransferase activity. DNA methyltransferase enzymes (DNMTs) recognize CpGs within double-stranded DNA as substrates. During embryogenesis, *de novo* methylation is performed by DNMT3A and DNMT3B. DNA methylation is generally maintained by DNMT1 and occurs in step with DNA replication. DNMT1 in turn passes on epigenetic control of gene expression to daughter cells. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)

one may consider one of the major roles of DNMT1 as passing on epigenetic control of gene expression to daughter cells (126). DNMT3A and DNMT3B are critical for *de novo* methylation during embryogenesis (Fig. 4) (100). Although some studies suggest an ongoing role for DNMT3A and DNMT3B in maintaining the methylation status in some cell types, the ubiquitously expressed DNMT1 is predominantly responsible for maintaining the cellular levels of CpG methylation (100).

Epigenetic alterations have been shown to be induced by the ROS, hydrogen peroxide (H_2O_2), where DNMT1 becomes more tightly bound to chromatin after H_2O_2 treatment and thereby alter the methylation status of CpG regions (98). Although not specifically studied in CVD, it is possible that ROS may influence the methylation status in atherosclerotic lesions in a similar fashion.

Mice deficient in genes coding for methylation enzymes, including DNMTs or methylenetetrahydrofolate reductase (MTHFR—related to methyl donor generation), show hypomethylation of their DNA. Increased expression of inflammatory mediators is found in leukocytes from DNMT-deficient mice (84). In MTHFR-deficient mice, DNA hypomethylation has been shown to precede the formation of aortic fatty streaks (26).

Atherosclerosis

Atherosclerosis is a dynamic process involving numerous cell types—monocytes, endothelial cells, smooth muscle cells (SMCs), and T-cells. A chronic inflammatory response with infiltration of macrophages and T-cells along with endothelial dysfunction is also important in the pathogenesis of plaque formation (83). In response to inflammation or injury, production of ROS is enhanced in vascular cells. These changes contribute to the initiation of atherosclerosis. Epigenetic changes have been detected in atherosclerotic lesions that may contribute to the pathogenesis of vascular lesions. DNA methylation has also been shown to control leukocyte functions that are related to cardiovascular risk, including the expression of soluble mediators and surface molecules that

direct margination, adhesion, and migration of blood leukocytes in vascular tissues (6).

SMCs play a major role in the atherosclerotic process. In advanced human atherosclerotic plaques, hypomethylation has been found in proliferating vascular SMCs (VSMCs). Hypomethylation has also been observed in the aortas of apolipoprotein E (ApoE) knockout mice as early as 4 weeks of age and before any histological atherosclerotic changes (132). One of the earliest studies linking DNA methylation to atherosclerosis showed that the extracellular superoxide dismutase (SOD) gene was hypomethylated in atherosclerotic lesions in rabbits (78).

Nitric oxide synthases (NOSs) are important in CVDs and are epigenetically modulated. NO plays an important role in the protection against and the progression of CVD. The underlying pathology for most CVDs is atherosclerosis, which is associated with endothelial dysfunction. The cardioprotective roles of NO include regulation of blood pressure and vascular tone, inhibition of platelet aggregation and leukocyte adhesion, and prevention SMC proliferation (65). Reduced bioavailability of NO is thought to be one of the central factors common to CVDs. Inactivation of the signaling molecule NO by ROS is recognized to be a key mechanism underlying reduced NO bioavailability and the development of endothelial dysfunction, which may itself be an important contributor to disease pathophysiology (52). The production of NO is catalyzed by three isoforms of NOS encoded by separate genes on three different chromosomes: neuronal NOS (*NOS1*), inducible NOS (*iNOS* or *NOS2*), and endothelial NOS (*eNOS* or *NOS3*). These three NOS isoforms differ in their regulation and cell-specific distribution. *eNOS* is constitutively expressed and responsible for the majority of NO produced by the vascular endothelium and, therefore, represents the major source of bioactive NO. Methylation plays a variable role in the various forms of NOS and in different tissues. *eNOS* is hypomethylated in the endothelium, but heavily methylated in SMC lines and other tissues (22,40). *iNOS* is expressed in atherosclerotic plaque neointima (82,137), but repressed by methylation in most tissues (22). Peroxynitrite (formed by the reaction of NO with superoxide) is increased in peripheral blood vessels during hyperhomocysteinemia (133). A reaction between free radicals such as superoxide and NO decreases NO availability and generates peroxynitrite, which enhances the cellular oxidative stress by increasing the endothelial dysfunction, and stimulating prothrombotic effects such as increased platelet reactivity, reduced endogenous fibrinolysis, and lipid peroxidation.

Estrogen receptors (ERs) are present in the coronary arterial wall on both SMCs and endothelial cells and may play an important role in protection against atherosclerosis (134). Estrogens appear to have protective effects against oxidative stress mediated by *ER-α*. Deficiencies in *ER-α* lead to accelerated atherosclerosis in men (90). *ER-α* has a varying degree of methylation throughout the cardiovascular system. In human atherosclerotic tissues, the *ER-α* promoter is hypermethylated in SMCs, but not in endothelial cells (109). Higher degrees of methylation were found in coronary atheromas when compared to normal tissues. *ER-α* promoter methylation has been demonstrated to increase with age even in normal tissues and reach nearly a complete methylation level in the elderly (109). The *ER-β* gene also displays a correlation in methylation of its promoter and the presence of

atherosclerosis (70). Contrary to ER- α expression, expression of ER- β correlates with atherosclerosis independent of age (29). The ER- β promoter shows a high level of methylation in cells from the plaque area when comparing plaque versus nonplaque tissue from the same vessels. Increased expression of ER- β was observed with *in vitro* assays in endothelial cells and SMCs after administration of a DNMT inhibitor, 5-aza-2'-deoxycytidine, and an HDAC inhibitor, trichostatin A (TSA) (70).

DNA methylation has been suggested to reflect altered functions of cell types involved in immune or inflammatory responses during atherosclerosis (141). Because of the established roles of inflammation and leukocytes in atherosclerosis, peripheral blood leukocytes represent a biologically relevant cell type for cardiovascular studies. Global DNA hypermethylation predisposes to atherosclerosis (119). This predisposition to atherosclerosis was found in analyzing patients with angiographically confirmed the presence of coronary artery disease (CAD) compared to healthy controls. Susceptibility of peripheral blood leukocyte DNA to methylation-sensitive restriction enzymes was found to be associated with a higher risk of developing atherosclerosis (119). The presence of inflammation associated with increased cardiovascular mortality rate in patients with end-stage renal failure on hemodialysis has been shown to be associated with global DNA hypermethylation of peripheral blood lymphocyte DNA (125). This association is not definitive, however, as contrasting studies found lower DNA methylation content in peripheral blood leukocytes from patients with atherosclerotic CVDs (20). Similarly, in a study of patients with chronic kidney disease, no association was found between global DNA methylation content in peripheral blood DNA and the intima media thickness, which is taken as a surrogate marker of subclinical atherosclerosis (95).

DNA methylation is believed to play important roles in the maintenance of genome integrity by transcriptional silencing of retrotransposons, retrovirus-like DNA sequences that can duplicate and transpose themselves across the genome. Retrotransposons, such as Alu and long-interspersed nucleotide elements-1 (LINE-1), have been extensively investigated in human epigenetics studies. Work in the Normative Aging Study, which consisted of men who received care in Veterans Administration hospitals, showed a longitudinal decline in the average blood genomic DNA methylation of repetitive sequences, such as Alu and LINE-1, over 8 years of follow-up (15). Genome-wide profiling of DNA methylation in blood DNA samples taken 11–16 years apart in recent studies from two cohorts from Iceland and Utah demonstrated both losses and gains in methylation over time, depending on the loci (12). Elevated, not decreased, Alu methylation in peripheral blood leukocytes has been shown to be related to the prevalence of CVD and obesity in Chinese individuals (71). However, global methylation measures provide average estimates of methylation across the entire genome or in wide portions of the DNA, such as repetitive elements, and as such, do not have the resolution necessary to pinpoint individual genes or sequences responsible for CVD risks. Decreased LINE-1 and Alu methylation may be accompanied by reactivation of different sets of silenced genes, which may be responsible for the opposite associations with the cardiovascular risk. Thus, while epigenetic regulation clearly occurs in atherosclerosis, there is a not-yet certainty about the relative importance of

global versus gene-specific changes in methylation, nor is it clear whether redox perturbations result from these epigenetic alterations, suggesting important areas for research.

Congestive Heart Failure

Heart failure is the result of a host of complex interactions involving the myocardium, vasculature, and neurohormonal systems. Oxidative stress is greatly increased in heart failure, but whether this is a cause or an effect has continued to undergo considerable scrutiny. Both *in vitro* and *in vivo* studies have shown that myocyte contractile function may be impaired by increased ROS through several mechanisms, including disruption of calcium cycling, altered myofilament responsiveness to calcium, and deleterious effects on cellular metabolism and energetics (44). In experimental settings, many studies have shown beneficial effects of antioxidants (*e.g.*, vitamin C and vitamin E), especially in the short term. However, larger prospective randomized trials in human subjects looking at hard cardiovascular endpoints have been disappointing. For example, neither the Heart Outcomes Prevention Evaluation Study nor the Heart Protection Studies found any evidence for a beneficial effect of antioxidants (1,140).

Gene expression reprogramming in cardiomyocytes has been identified in cardiac hypertrophy and failure and is characterized by upregulation of fetal genes, known as the fetal gene program. While the role of histone modifications such as histone acetylation and miRNA function (130) in cardiac hypertrophy and failure has been more extensively investigated, little is known about the involvement of DNA or histone methylation in these processes.

In left ventricular samples obtained at the time of cardiac transplantation, changes in DNA methylation were shown to correlate with the gene expression of angiogenic factors, namely, the endothelial cell marker, the *PECAM1*, *ARH-GAP24*, and *AMOTL2* gene (93). A recent study explored the global epigenomic profiles in human cardiomyopathy through generation of genome-wide maps of DNA methylation and H3K36me3 enrichment. DNA methylation was found to differ between end-stage cardiomyopathic hearts and control human hearts in the CpG islands globally within gene promoters and gene bodies, but not in intergenic CpG islands or in CpG islands within the 3'-untranslated region. A significant decrease in global gene promoter methylation also correlated with the genes that were upregulated in cardiomyopathy, but not with the genes that were downregulated (94). However, alterations in redox signaling from epigenetic changes have not yet been studied in heart failure.

Hypertension

Patients with hypertension demonstrate increased levels of oxidative stress byproducts, increased oxidative DNA damage, and decreased activity of endogenous antioxidant enzymes in blood and mononuclear cells when compared to normotensive individuals (112). Vascular ROS production is also elevated in a range of different experimental models of hypertension, including Ang II-induced, mineralocorticoid, and renovascular hypertension. Thus, there is compelling evidence to suggest a role for ROS in the pathogenesis of hypertension. Recent research has identified the targets for the

environment–gene interactions in various hypertensive disorders and possible evidence for epigenetic contributions to hypertension. Analysis of peripheral blood leukocytes of patients with hypertension has shown a loss of global genomic DNA methylation content (123). Changes in the DNA methylation status occur in the genes implicated in the development of hypertension. The proximal promoter of the *AT1b* angiotensin receptor gene in the rat adrenal gland has been found to be significantly hypomethylated compared to controls, and that *AT1b* gene expression is highly dependent on promoter methylation. When expression of the *AT1b* gene in the adrenal gland is upregulated by hypomethylation during the first week of life, increased expression of this receptor protein increases the responsiveness of the adrenal gland to angiotensin (14). This scenario may contribute to an exaggerated response to salt and salt-sensitive hypertension by epigenetic mechanisms.

Cell culture and animal studies have suggested a role for gene regulation *via* methylation of the promoter of the *HSD11B2* gene in the development of hypertension. Lower activity of *HSD11B2* classically induces hypertension by leading to an altered THF/THE shuttle. This results in cortisol activating mineralocorticoid receptors and sodium retention. Patients with essential hypertension who have an elevated urinary THF/THE ratio also show a higher *HSD11B2* promoter methylation (42,123).

In pre-eclampsia, the main hypertensive disorder of pregnancy, epigenetic changes are observed within the placenta with differences in the expression profiles of endogenous serine protease inhibitors. The promoter regions of genes coding for 10 such endogenous serine protease inhibitors are either totally methylated or totally unmethylated in the placentas of patients with pre-eclampsia (24). The serine protease inhibitor *A3* gene is significantly hypomethylated in the placentas from pregnancies complicated by pre-eclampsia and may provide a potential biomarker for pre-eclampsia (24). The relevance to ROS signaling; however, has yet to be defined.

Pulmonary Hypertension

Pulmonary hypertension (PH) is a cardiopulmonary disease in which the mean pulmonary artery pressure at rest exceeds 25 mm Hg. There are five groups of PH recognized in the current classification. In Group 1 PH, the elevated pulmonary arterial (PA) pressure reflects vascular obstruction due to obliteration and constriction of small pulmonary arteries. In this type of PH, also known as pulmonary arterial hypertension (PAH), there is evidence for an epigenetic contribution to the hyperproliferative phenotype of SMCs. Epigenetic regulation of gene expression may link known risk factors for the development of PH and contribute to the net risk of developing overt disease as well as play a role in the phenotypic variability of PAH, such as severity and time to disease onset (69). SODs are a ubiquitous family of enzymes that function to efficiently catalyze the dismutation of superoxide anions. Epigenetic regulation of the mitochondrial enzyme SOD2 provides an example where epigenetic derangement of redox signaling contributes to the hyperproliferative phenotype of pulmonary artery SMCs (PASMCs). SOD2 deficiency has been identified in the pulmonary arteries and plexiform lesions of PAH (16,17), and activation of HIF-1 α is also evident (5,16,17,36,38). However, the mechanism

by which these two abnormalities were linked had been unclear. In fawn-hooded rats, the development of PAH is preceded by downregulation of SOD2, and the selective hypermethylation of CpG islands in the *SOD2* gene reduces its expression ~50% compared to PASMCs from genetically matched controls (16). This decreased SOD2 expression reduces production of H₂O₂, which is involved in pulmonary vascular oxygen sensing and regulation of proliferative, antiapoptotic sensitivity of PASMCs (5). Epigenetic silencing of SOD2 promotes SMC proliferation, which is restricted to the pulmonary circulation (leaving systemic arteries unaffected).

Both DNMT1 and DNMT3B are significantly upregulated in lung tissue and PASMCs compared to control. Treatment with the DNMT inhibitor, 5-azacytidine (5-AZA), results in a dose-dependent increase in SOD2 expression and reduced SMC proliferation (5). SOD2 methylation is important in the development of PAH and is one factor that contributes to HIF-1 α activation and the development of a proliferative, apoptosis-resistant state (5). The altered redox state resulting from SOD2 downregulation and subsequent HIF-1 α reduction contributes to a glycolytic shift in lung metabolism (85). It remains uncertain how DNMT expression is selectively increased in pulmonary versus systemic arteries in PAH. However, the plasticity of the phenotype suggests the possibility for an epigenetic-based PAH therapy. Challenges remain, particularly the current difficulty of targeting one or a few genes for selective modulation.

Environment and Nutrition

The development of the embryo occurs in a relatively low-oxygen environment. It is highly sensitive to injury to oxidant molecules because of its low antioxidant capacity (33). ROS serve as signaling molecules that induce transcription of several genes (*e.g.*, *HIF-1 α* , *CREB1*, *NFKB1*, and *NRF2*) important in oxygen sensing, cell differentiation, and proliferation. Oxidant molecules can directly interact with DNA base pairs causing both genetic, as well as, epigenetic changes (21).

The Barker hypothesis suggests that fetal stressors can lead to CVDs in adults (7). Groundbreaking work elucidating this mechanism in humans came from Heijmans and coworkers (53,129), who studied the individuals prenatally exposed to famine during the Dutch Hunger Winter in 1944–1945. Compared to siblings of the same sex, these individuals displayed an altered genetic methylation pattern, a condition that has been associated with obesity, impaired glucose homeostasis, and increased cardiovascular risk in adulthood. DNA exhibited hypomethylation of the imprinted *IGF2* and *INSIGF* genes and hypermethylation of the *GNASAS*, *MEG3*, *IL10*, *ABCA1*, and *LEP* genes compared to unexposed siblings (128). The prevalence of obesity and coronary heart disease in these individuals was higher than that of adults born before or conceived after that period (102).

Animal models designed to evaluate the epigenetic mechanisms of intrauterine programming provide more concrete data in the context of controlled dietary interventions. Alkemade *et al.* demonstrated that the ApoE + / – offspring of ApoE – / – hypercholesterolemic mice were more susceptible to postnatal dietary-induced atherosclerotic changes than genetically similar offspring born to wild-type mothers. Moreover, their carotid endothelial and VSMCs displayed altered histone methylation and lysine methyltransferase expression (2).

While the adverse effect of a poor maternal diet and low birth weight on the lifetime development of CAD is recognized (102), the effects of maternal malnutrition on the right ventricle and PH have only recently been considered. Rexhaj *et al.* assessed the pulmonary vascular responsiveness in offspring of pregnant mice fed with a restrictive diet in both normoxic and hypoxic conditions (114). To detect reversible epigenetic changes, the authors also administered the aforementioned HDAC inhibitor TSA to male offspring of pregnant mice that had been fed a restrictive diet.

In the offspring of restrictive-diet pregnancy, there was exaggerated hypoxic PH and right ventricular hypertrophy (RVH). An epigenetic change was demonstrated by the increased uptake of radioactive methyl groups in the offspring of the pregnant diet-restricted mice. The pulmonary DNA methylation induced by dietary restriction and the epigenetic changes were reversed by the administration of HDAC inhibitors, butyrate and TSA. To investigate the role of increased oxidative stress during restrictive-diet pregnancy, the authors gave the nitroxide, Tempol, to the mother during restrictive-diet pregnancy and found that Tempol prevented pulmonary vascular dysfunction in the offspring and prevented DNA demethylation.

It is clear that unfavorable changes in dietary habits and smoking contribute to atherosclerosis *via* increases in blood lipid levels and uptake of cholesterol in the arterial wall. In addition, dietary modification can affect the methylation machinery in the arterial cells, thereby initiating epigenetic modifications of the genome that promote atherogenesis (54). In the setting of cigarette smoking, free radical-mediated oxidative stress could arise from (i) the gas or tar phase of cigarette smoke; (ii) circulating or *in situ* activated macrophages and neutrophils; and (iii) endogenous sources of ROS such as uncoupled eNOS, xanthine oxidase, and the mitochondrial electron transport chain (96). A reaction between free radicals such as superoxide and NO not only decreases NO availability but also generates peroxynitrite, which further enhances the cellular oxidative stress. Increased oxidative stress with the loss of the protective effect of NO tips the cellular balance toward a proatherogenic and prothrombotic milieu (74,117).

The dynamic changes in DNA methylation appear to be influenced by additional factors related to the cardiovascular risk. Three independent studies consistently demonstrated that exposure to air pollution, an established risk factor for ischemic heart disease and stroke, is associated with reduced blood methylation of LINE-1 (6). Using a candidate gene approach, hyper- and hypomethylation of specific genes were shown to be related to air pollutant exposures, including increased *CDKN2B* methylation and decreased *iNOS* and *MAGEA1* methylation (6). Recently, Breton *et al.* showed that second-hand smoke induced lower Alu and LINE-1 DNA methylation and changes in methylation of specific genes in buccal mucosa samples obtained from children (18). These studies suggest that DNA methylation may be one of the mechanistic links between exposure to the pollutants and the development of CVD.

It has been shown that a maternal diet low in protein is associated with reduced global methylation. It is hypothesized that the reason for such changes is a deficiency of specific amino acids, which are required to generate methyl donors (113). The methyl group responsible for DNA and histone methylation originates from *S*-adenosyl methionine

FIG. 5. Homocysteine reactions relevant to epigenetic marks.

The main source of methyl groups for hundreds of trans-methylases that methylate DNA, RNA, histones, other proteins, and small biological molecules is *S*-adenosylmethionine. Homocysteine can enter into several pathways, including methyltransferases, transsulfuration, or conversion into methionine. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)

(AdoMet) *via* Met biosynthesis through folate-dependent or folate-independent pathways of homocysteine remethylation. The methyl group responsible for the establishment and maintenance of DNA methylation patterns originates from AdoMet, an intermediate in homocysteine metabolism. Homocysteine is biochemically linked to the principal epigenetic tag found in DNA. DNA hypomethylation is induced by increases in homocysteine and *S*-adenosylhomocysteine, an intermediate in homocysteine metabolism and inhibitor of methylation (35). Global or selective DNA methylation may contribute to alterations in gene expression and vascular changes during hyperhomocysteinemia. A positive correlation has been found between global DNA methylation and homocysteine levels (Fig. 5). Homocysteine is known to be an independent risk factor for CAD (55). Patients with vascular disease manifest increased levels of both plasma homocysteine and intracellular Ado-homocysteine, together with decreased DNA methylation, which supports a role for hyperhomocysteinemia in modulating epigenetic mechanisms. Castro *et al.* demonstrated that patients with vascular disease have a disturbed global DNA methylation status associated with increased plasma homocysteine levels. High blood homocysteine levels correlate with DNA hypomethylation and atherosclerosis and can lead to a 35% reduction in the DNA methylation status of peripheral blood lymphocytes (20). This association has also been confirmed in several animal studies in which increased circulating levels of homocysteine and AdoHcy are associated with endothelial dysfunction and aberrant DNA methylation patterns (34,81). In contrast to these findings, Sharma and coworkers also observed a significant positive correlation of global DNA methylation with plasma homocysteine levels in CAD patients and concluded that alteration in genomic DNA methylation and the association with CVD appear to be further accentuated by higher homocysteine levels (120). Sharma *et al.* reviewed literature of 135 genes, which either modulate the

blood level of homocysteine or are regulated by the elevated level of homocysteine. Mapping of these genes to their respective pathways revealed that an elevated level of homocysteine may lead to atherosclerosis either by directly affecting lipid metabolism and transport or by oxidative stress and endoplasmic reticulum stress by decreasing the bioavailability of NO and modulating the levels of other metabolites, including *S*-adenosyl methionine and *S*-adenosyl homocysteine (120).

Although increased circulating levels of homocysteine are a risk factor for vascular disease, recent clinical trials that used folate or other vitamin B therapies to lower homocysteine failed to reduce the cardiovascular event rates, thus casting a doubt on the direct causative role of homocysteine in vascular disease (3,8). The reduction in homocysteinemia and the increased availability of methyl compounds provided by vitamin supplementation, such as folic acid, may not be sufficient to affect genomic DNA methylation (107). Aberrant global DNA methylation is only an index of the potential for epigenetic dysregulation. In addition to AdoHcy, a growing list of factors have been identified that can modify the DNA methylation patterns. These include the rate of cell growth and DNA replication, chromatin accessibility, local availability of AdoMet, nutritional factors (including folate supplementation), duration and degree of the hyperhomocysteinemic state, inflammation, dyslipidemias, oxidative stress, and aging (58). The relationship between increased Hcy and DNA global hypomethylation may be masked in the clinical setting owing to the presence of these confounders, thereby possibly explaining some contradictory and counterintuitive findings reported to date. Another important aspect to consider is that DNA

methylation is unequally distributed throughout chromosomes of differentiated cells, and hypermethylated and hypomethylated regions can coexist in the genome. The global DNA methylation status need not correspond to the methylation status of specific genomic regions.

Histone Modification

Besides methylation of DNA, post-translational modifications of N-terminal tails of histone proteins are key components in the epigenetic regulation of genes. Histone modifications and DNA methylation provide a close interplay with respect to gene regulation as both activities are functionally linked. Histone modifications change DNA accessibility, and this modulates the expression of genetic information. Epigenetic modification of histone tails occurs through methylation, acetylation, SUMOylation and ubiquitination of lysine residues, methylation of arginines, and phosphorylation of serine residues. A code is formed by the histone modifications that when read has different effects on gene accessibility and chromatin structure. Modifications of histone tails include (among others) acetylation and methylation of lysine residues. While acetylation of histone tails is correlated with gene activation, the influence of histone methylation depends on the exact residue methylated and the number of added methyl groups. Lysine methylation and acetylation are the most studied modifications, but there are many more histone modifications known (Fig. 6) (60,116). Euchromatin, a conformationally relaxed chromatin, is a feature of potentially active disease and is associated with acetylated histones. Nonacetylated histones have been

FIG. 6. Post-translational modifications of histones. The site of action of epigenetic histone modification. Nucleosomes package ~146 base pairs of DNA wrapped around an octamer of core histone proteins. Each core nucleosome consists of two of each histone protein: H2A, H2B, H3, and H4. The modifications include acetylation (Ac), methylation (Me), phosphorylation (P), and ubiquitination (ub).

observed in compact chromatin (heterochromatin) and are seen in transcriptionally silent genes with DNA hypermethylation at CpG dinucleotides. DNMTs, histone methyltransferases (HMTs), and histone acetyltransferases (HATs) mediate the effects on chromatic modifications.

HATs and HDACs potentially play a role in atherosclerosis and restenosis after coronary intervention by promoting neointima formation, SMC proliferation, and inflammation (108). HATs transfer acetyl groups to the epsilon-amino-portion of the lysine residues resulting in regulation of gene expression by acetylation of core histones. VSMCs are phenotypically effected by atherosclerosis and intimal injury, and a part of that is mediated through histone modifications (87). Some of this effect is achieved through serum response factor (SRF). SRF binds to the CArG-box DNA sequences, which activate genes that are responsible for SMC-restricted expression. SRF causes post-translational histone modifications such as acetylation of histone H4, acetylation of lysine 9 in histone H3, and dimethylation of lysine 4 and 79 in histone H3. These changes affect the SMC lineage (88) and suppress SMC differentiation in response to vessel injury. In comparison with normal coronaries, there is increased histone acetylation present in VSMCs from coronary atherosclerotic lesions. This increase is mediated by hypernuclear acetylation (HNA), which impacts apoptosis and proliferation (67). The effects of HNA on VSMCs are further increased by thrombin mediated through MAP kinase (68).

Histone modifications have been demonstrated in animal models of atherosclerosis and inflammation. In diabetic vascular disease, altered histone modifications may mediate the metabolic changes and proinflammatory alterations in SMCs (132). Not only is cytokine expression under epigenetic control, but cytokines themselves also induce (indirect) changes to the chromatin, providing an essential link between inflammation and epigenetic programming (89).

There is evidence showing the importance of histone acetylation during cardiac hypertrophy and failure. Increased histone dimethylation and acetylation are observed when there is increased atrial natriuretic peptide and brain natriuretic peptide expression in the left ventricle, and this is mediated through histone acetyltransferase (86). Histone methylation plays a significant role in the physiological function of cardiomyocytes. Animal models of heart failure have revealed that cardiomyocytes of failing hearts have a differential marking of trimethylation of H3K4 and H3K9. This has also been observed in humans with heart failure (66). Trimethylation of lysine 4 of histone H3 results in a decrease in the levels of *Kcnip2*. This results in an impaired cellular function affecting sodium and L-type calcium current, as well as prolonged duration of action potential. Reducing histone H3 lysine 4 methylation in differentiated cardiomyocytes results in deletion of PTIP protein. This increases the sensitivity of cardiomyocytes to premature ventricular complexes (124). In patients with advanced heart failure, epigenomic patterns have been observed (94), such as differential DNA methylation and trimethylation of lysine 36 of histone 3. These studies do not address whether changes in the redox status occur (49,88).

Sirtuins are a highly conserved family of histone/protein deacetylases and have been shown to participate in biological functions related to the development of heart failure, including regulation of energy production, oxidative stress, intracellular signaling, angiogenesis, autophagy, and cell death/

survival. Emerging evidence indicates that two sirtuins (SIRT1 and SIRT3) play protective roles in failing hearts (127). The poly-ADP ribose polymerases (PARPs) utilize nicotinamide adenine dinucleotide (NAD^+) to fix complex polymers of poly(ADP-ribose) to protein targets, including PARP1 and many of the core histone proteins. PARPs depend on the local concentrations of NAD^+ for appropriate functionality, setting up a direct competition between PARP and sirtuin activities in nuclear settings. This is a particularly important relationship due to the generally opposed natures of sirtuin and PARP modifications to histones; while PARP is associated broadly with chromatin relaxation, histone acetylation, and minimal DNA methylation, sirtuins are broadly associated with the removal of the permissive histone acetylation mark, promoting gene silencing and chromatin compaction (50). Supporting the importance of these competing activities, multiple groups have identified regulatory interactions between PARP and sirtuin enzymes. Pillai *et al.* demonstrated that PARP-1 functionally depletes the NAD^+ pools, attenuating the nuclear sirtuin activity in a model of cardiac myocyte cell death in heart failure (105). The antihypertrophic effects of exogenous NAD was shown to be mediated through activation of SIRT3, but not SIRT1, thus revealing a novel role of NAD as an inhibitor of cardiac hypertrophic signaling, and suggest that prevention of NAD depletion may be critical in the treatment of cardiac hypertrophy and heart failure (106).

While the pathogenesis of ischemia and reperfusion is not completely understood, there is considerable evidence implicating ROS as an initial cause of the injury. In this area, caveolin plays an important role (131), and caveolin knockout mice have provided insight into histone methylation in this model. After ischemia/reperfusion in caveolin knockout mice, there is an increase in histone methylation. This increase in histone methylation is associated with an increase in the HDAC activity and an elevated level of HMT G9a protein. There is a decreased expression of sirtuin-1 observed in caveolin knockout mice and a reduction in the translocation of Foxo-3a to the nucleus. Further supporting the cardioprotective role caveolin, the caveolin knockout mice had decreased ventricular function and increased apoptosis of cardiomyocyte cells in the setting of ischemia and reperfusion (131).

In a study by Xu *et al.*, the authors demonstrated a sixfold upregulation of eNOS expression in pulmonary vascular endothelial cells derived from a neonatal rodent-persistent PH of the newborn model induced by intrauterine exposure to hypoxia and indomethacin between the 19th and 21st day of gestation. In this model, NOS upregulation was associated with an increased H3 and H4 histone acetylation in the eNOS promoter (138). They also noted a mild decrease in eNOS methylation.

Hypoxia has major effects on the endothelial phenotype. In general, hypoxia decreases the global transcriptional activity. The HIF transcription program allows cells to adapt to changes in oxygen supply or availability. Evidence suggests that epigenetic pathways may be relevant in this pathway. Hypoxia induces a global decrease in H3K9 acetylation in various cells as a possible consequence of HDAC upregulation (62). Under hypoxic conditions, increased global H3K9 methylation has been observed across different cells and is attributed, in part, to increased G9a HMT expression (25). Hypoxia decreases expression of eNOS *via* transcriptional repression. Hypoxia causes a rapid and sustained decrease in H3/H4 acetylation of eNOS proximal promoter histones. Surprisingly,

this is mediated *via* histone eviction from the *eNOS* proximal promoter during hypoxia. This is followed by the subsequent reincorporation of histones that lack H3/H4 acetylation (41). Angiogenesis can also be impacted by epigenetic silencing of the *eNOS* promoter through methylation of the lysine residue 27 on histone 3. This epigenetic silencing is decreased during hypoxia, and thus *eNOS* expression is increased (99). This demonstrates that histone modifications can be dynamically regulated by cellular oxygen content and therefore highly relevant to diseases of the cardiovascular system.

RNA Interference

miRNAs are endogenous, conserved, small, noncoding RNAs, which regulate target messenger RNA (mRNA) expression. miRNAs are situated in the intergenic regions of the genome or are coexpressed with a host gene transcript and regulate the critical aspects of cardiac function as well as play a role in CVD and development [79]. miRNAs interact with mRNA through sequence-specific interactions with key areas of sequence homology at the 5'-end of the miRNA, particularly at bases 2–8, which is termed the seed region (Fig. 7). This control is sequence specific, and changes in just a single base within the miRNA target site can abolish this regulation. It is this region that forms the basis for virtually all computational algorithms predicting the mRNA targets. However, beyond Watson–Crick base pairing, the efficiency of repression depends on the number and configuration of mismatches between the miRNA and

the target mRNA, the secondary structure of the surrounding region, and the number of target sequences on the mRNA (56). Up to one-third of human genes are likely regulated by one or more miRNAs. Despite this vast regulatory network and hundreds of miRNAs, few targets have been validated (61).

ROS have been implicated to serve as signaling molecules in numerous mechanisms, including angiogenesis. NADPH oxidase is one of the major sources of ROS in vasculature. The superoxide-generating NADPH oxidase Nox2 with the subunit p22^{phox} is converted to an active state by the assembly of a membrane-localized cytochrome b₅₅₈. These membrane components are associated as a part of a multicomponent enzyme system that minimally requires 3 cytosolic components: p47^{phox}, p67^{phox}, and Rac1. Evidence that redox signaling in cells is subject to regulation by miRNA was shown through Dicer knockdown in human microvascular endothelial cells. This demonstrated lower inducible production of ROS when activated with phorbol ester, tumor necrosis factor- α , or vascular endothelial growth factor. The miRNA deficiency caused by Dicer knockdown specifically downregulated both p47^{phox} expression as well as ROS production. Thus, p47^{phox} of the NADPH oxidase complex has been identified as a target of miRNAs (121).

Long noncoding RNAs are defined as noncoding transcripts >200 nucleotides long. They are classified as sense, antisense, bidirectional, intergenic, or intronic, depending on their position [19]. Long noncoding RNAs have been studied in VSMCs. sONE is an antisense transcript to *eNOS*, and

FIG. 7. Schematic of miRNA biogenesis and function. miRNA transcription is mediated by RNA polymerase II, resulting in pri-miRNAs. Pri-miRNAs are subsequently processed by Drosha into pre-miRNAs. The pre-miRNA, ~70 nt in length, is exported into the cytoplasm where Dicer, an RNase III, cleaves the pre-miRNA into a miRNA duplex. After incorporation into an RISC complex, the mature miRNA is formed and directed to its proper mRNA targets. miRNAs interact with mRNA through sequence-specific interactions with key areas of sequence homology at the 5'-end of the miRNA, particularly at bases 2–8 termed the seed region.

when inhibited through RNA interference, is decreased in expression in VSMCs, resulting in increased eNOS expression. In turn, eNOS expression is decreased with overexpression of SONE in endothelial cells (115).

Not only can classical DNA methylation alter miRNA levels, but the reverse can also occur. A subset of miRNAs controls the expression of important epigenetic regulators, including DNA methyltransferases and HDACs (30). miRNA-29b can reduce the expression of DNMT enzymes and thereby affect the global methylation status (45). Wang *et al.* demonstrated that miRNA-152 can knockdown DNMT1 in human aortic SMCs, leading to hypermethylation of the *ER-α* gene (135). HDAC expression can be regulated by miRNAs, such as miRNA-449a (97).

Defining the epigenetic role of miRNAs in CVD is still at an early stage. miRNA-33 modulates cellular cholesterol synthesis and biogenesis of high-density lipoprotein (HDL) in the liver (111). miRNA-33 predominantly targets the gene encoding the ATP-binding cassette transporter *ABCA1*, which is involved in cellular cholesterol mobilization. *ABCA1* protein levels are reduced by overexpression of miRNA-33. Antagonism of endogenous miRNA-33 through miRNA-33-antisense oligonucleotides increases cholesterol efflux and *ABCA1* protein levels. Lentiviral delivery of anti-miRNA-33 increases plasma HDL levels. Downregulation of miRNA-204 (31) and increases in miRNA-21 (103) have been identified as potential mediators of PAH. Finally, it has been demonstrated that a specific set of miRNA molecules are upregulated by hypoxia. In particular, the HIF-responsive miRNA-210 was shown to be ubiquitously expressed in the hypoxic cell and tissue types (23).

It is becoming evident that a complex network exists between miRNAs and epigenetic pathways to form an epigenetic-miRNA regulatory circuit, which in turn may act to organize the whole-gene expression profile (30). Additional studies are clearly needed to further elucidate how epigenetic phenomena impact miRNAs important in CVD as well as how miRNAs may impact the epigenetic marks of genes pertinent to CVD. Although the redox contribution of several of these miRNAs are unclear, further study is warranted as the field of epigenetics evolves.

Alteration of Epigenetic Modifications

The reversible nature of these epigenetic modifications makes the chromatin-modifying enzymes interesting therapeutic targets. The functional importance of DNA methylation and histone post-translational modifications at the *eNOS* promoter was demonstrated by pharmacological inhibition studies. Treatments of VSMCs with 5-AZA and TSA upregulated the *eNOS* mRNA levels (43). In contrast, the H3K4 methylation inhibitor, methylthioadenosine, downregulates eNOS expression in endothelial cells (40).

There has been some recognition that one effect of statin therapy is the alteration of epigenetic mechanisms. TSA and lovastatin inhibit HDAC activity. Both TSA and valproic acid lowered plasma cholesterol levels in low density lipoprotein (LDL) knockout mice by increasing CYP7A1 expression and bile acid synthesis (92). Treatment with TSA also accelerates macrophage infiltration and exacerbates neointimal lesions in *LDLR^{-/-}* mice and underscores the need for improving our understanding of the epigenetic pathways in CVDs (28,132).

Cho *et al.* studied the effects of an HDAC inhibitor, sodium valproate, on RVH in monocrotaline (MCT) rats and pulmonary artery-banding (PAB) rats. MCT rats develop a maladaptive form of RVH and tend to die within 6 weeks with right ventricle (RV) failure; conversely, PAB rats develop an adaptive form of RVH and are much less prone to premature death or RV failure (27). HDAC inhibition in PAB rats significantly decreased RVH. Pulmonary artery flow acceleration was significantly reduced in PAB rats treated with sodium valproate, suggesting a functional as well as anatomical improvement with an epigenetic modification. HDAC inhibition also reduced myocardial fibrosis. The involvement of HDACs in these responses was demonstrated by the increased acetylation of histone H3 in the RV in animals that received sodium valproate versus controls. As would be expected with sodium valproate, there was no change in HDAC1, HDAC2, HDAC3, and HDAC8 expression. Similar improvements in RVH were observed in MCT rats treated with sodium valproate.

FIG. 8. A conceptual model linking epigenetics to cardiovascular disease.

Not all studies suggest benefits from HDAC inhibition in RVH. In a study by Bogaard *et al.* (13), TSA was administered intraperitoneally 4 weeks after PAB. TSA-treated rats developed decreased cardiac output and more signs of RV failure. In contrast to the Cho study, TSA increased RVH in PAB. TSA-treated PAB rats also demonstrated increased RV fibrosis, capillary rarefaction, and rates of cell death. These negative results also contrast with the beneficial effects of HDAC inhibition in left ventricular (LV) hypertrophy (75). Do these differences reflect the differences between HDAC inhibitors, differences in the models, or (in the case of left ventricular hypertrophy) differences in the RV versus the LV transcriptome response to pressure overload? (76) There are several classes of HDAC inhibitors, and further study is clearly required to determine whether a subclass-specific inhibition of a specific HDAC family is beneficial or harmful.

Conclusions

Epigenetic patterns differ in patients with CVD compared to controls. Whether epigenetic changes are causally related to pathogenetic features or whether they merely represent a consequence of the ongoing pathological process remains unclear. Environmental and genetic effects can only explain a small part of the variability in the CVD risk, even for well-established risk factors. Epigenetic regulation of gene expression may link known risk factors for the development of CVDs and contribute to the net risk of developing overt disease. Epigenetic changes may be the missing link between genomic sequence variation, comorbid disease states, environmental exposure, and cell-signaling events (Fig. 8).

Epigenetic modifications may be reversible, and it is possible that epigenetic modifications could be targeted by a pharmacological intervention. Inhibition of DNMTs or HDACs has shown both promise and harm in models of disease, as does the ability to inhibit miRNA. The lack of specificity for epigenetic treatments is an important issue and warrants a cautious application, given the concerns for off-target effects of HDAC inhibition or demethylating agents.

An increased understanding of epigenetic mechanisms may help lead to newer therapeutics as well as a new understanding of current therapies and their epigenetic effects. Additional studies are clearly needed to further elucidate how epigenetic phenomena impact on the development of CVD, and, in particular, the impact on the oxidative pathways. As technology to study epigenetics continues to advance, so must the understanding of the complex epigenetic map in both health and disease.

Acknowledgments

This work is supported by National Institutes of Health (NIH) K08-HL098565 (G.H.K.), NIH-RO1-HL071115 (S.L.A.), and 1RC1HL099462-01 (S.L.A.), the American Heart Association.

Author Disclosure Statement

No competing financial interests exist.

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Date of first submission to ARS Central, September 05, 2012; date of final revised submission, December 24, 2012; date of acceptance, January 15, 2013.

Abbreviations Used

5-AZA = 5-azacytidine
 ABCA1 = ATP-binding cassette transporter-1
 AdoMet = S-adenosyl methionine
 AMOTL2 = Angiomotin-like-2
 ApoE = Apolipoprotein E
 ARHGAP24 = Rho GTPase-activating protein 24
 CAD = coronary artery disease
 CDKN2B = cyclin-dependent kinase inhibitor 2B
 CpG = cytosine paired with guanine
 CVDs = cardiovascular diseases
 DNMTs = DNA methyltransferase enzymes
 eNOS = endothelial NOS
 ER = estrogen receptor
 GNASAS = GNAS antisense RNA
 H₂O₂ = hydrogen peroxide
 H3K36me3 = histone-3 lysine-36 trimethylation
 HAT = histone acetyltransferase
 HDAC = histone deacetylase
 HDL = high density lipoprotein

HIF-1 α = hypoxia inducible factor-1 α
 HMT = histone methyltransferase
 HNA = hypernuclear acetylation
 HSD11B2 = 11 beta-hydroxysteroid dehydrogenase-2 gene
 IGF2 = insulin-like growth factor II
 IL10 = interleukin-10
 iNOS = inducible NOS
 Kcnip2 = Kv channel-interacting protein 2
 LDL = low density lipoprotein
 LEP = leptin
 LINE-1 = long-interspersed nucleotide elements-1
 LV = left ventricular
 MAGEA1 = melanoma-associated antigen-1
 MCT = monocrotaline
 MEG3 = maternally expressed 3
 miRNAs = microRNAs
 mRNA = messenger RNA
 MTHFR = methylenetetrahydrofolate reductase
 NAD⁺ = nicotinamide adenine dinucleotide
 NIH = National Institutes of Health
 NOS = nitric oxide synthase
 PAB = pulmonary artery banding
 PAH = pulmonary arterial hypertension
 PARP = poly-ADP ribose polymerase
 PSMCs = pulmonary artery SMCs
 PH = pulmonary hypertension
 PTIP = PAX-interacting protein 1
 RISC = RNA-induced silencing complex
 ROS = reactive oxygen species
 RV = right ventricle
 RVH = right ventricular hypertrophy
 SMCs = smooth muscle cells
 SOD2 = superoxide dismutase 2
 SRF = serum response factor
 TF = transcription factor
 THFs/THE = tetrahydrocortisol-versus-tetrahydrocortisone metabolites
 TSA = trichostatin A
 3'UTR = 3'-untranslated region
 VSMCs = vascular smooth muscle cells