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# Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series)

Joseph Loscalzo, Diane E. Handy

Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

**Abstract:** Epigenetics refers to heritable traits that are not a consequence of DNA sequence. Three classes of epigenetic regulation exist: DNA methylation, histone modification, and noncoding RNA action. In the cardiovascular system, epigenetic regulation affects development, differentiation, and disease propensity or expression. Defining the determinants of epigenetic regulation offers opportunities for novel strategies for disease prevention and treatment.

**Keywords:** methylation, histone, noncoding RNAs, atherosclerosis, homocysteine, pulmonary hypertension.

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Epigenetics was first defined as the complex interplay between the genome and environmental factors that govern cell and organ differentiation and development. At the current time, this term refers to heritable traits that are not a consequence of changes in DNA sequence. These traits are the result of alterations in gene expression regulated by changes in DNA accessibility or chromatin structure. Epigenetic modifications, or tags, that lead to changes in DNA accessibility can be brought about by DNA methylation, posttranslational modification of histone proteins, or noncoding RNA actions in the nucleus.<sup>1</sup> Epigenetic modifications can be affected by exogenous factors and environmental exposures, providing a mechanistic link between genes (or the genome) and environment (or the exposome) in defining phenotype and offering an explanation for phenotypic differences between monozygotic twins.<sup>2</sup> The field of epigenetics is expanding rapidly, with a number of ongoing international research initiatives, including the Human Epigenome Project<sup>3</sup> and the International Human Epigenome Consortium.<sup>4</sup> Growing evidence supports the view that epigenetic modifications also contribute to the pathogenesis of cardiovascular disease and the expression of cardiovascular pathophenotypes.

## CHROMATIN STRUCTURE

Chromatin comprises nuclear DNA associated with specific nuclear proteins. The DNA coils around histone pro-

teins forming nucleosomes; the nucleosome contains an octomeric core of histone proteins (two H3-H4 histone dimers and two H2A-H2B dimers) bound to a 147-base-pair stretch of DNA. The H1 histone protein binds to the internucleosomal DNA linker sequences. Chromatin structure is determined by nucleosome spacing and can be categorized as transcriptionally inactive, densely packed heterochromatin, or transcriptionally permissive, less densely packed euchromatin.<sup>5,6</sup> The structure of chromatin and its consequences for gene expression are regulated by biochemical modifications both to DNA and to the amino-terminal histone tails that extend from the nucleosome into the nuclear lumen.

## EPIGENETIC TAGS

### DNA methylation

Covalent attachment of a methyl group to the C5 position of cytosine comprises the principal epigenetic modification of DNA. This modification occurs primarily in CpG dinucleotide-containing regions, often in regulatory sequences that suppress gene expression. CpG methylation is important for transcriptional repression of transposons and repeat elements, for imprinting and X-chromosome inactivation,<sup>7</sup> and for tissue-restricted gene expression during development and differentiation.<sup>8</sup> Methylation of cytosines not within CpG sequences can also occur and is important for regulation of gene expression in embryonic stem cells.<sup>9</sup>

Address correspondence to Dr. Joseph Loscalzo, Department of Medicine, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115-2394, USA. E-mail: jloscalzo@partners.org.

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The presence of a methylated cytosine can repress transcription by inhibiting the binding of transcription factors or may promote the binding of other transcriptional repressors, including histone-modifying proteins, such as histone deacetylases (HDACs). Cytosine methylation at CpG dinucleotides is carried out by a family of enzymes, the DNA methyltransferases (DNMTs), which include the de novo methyltransferases DNMT3a and DNMT3b, and by DNMT1, which recognizes and methylates the nonmethylated daughter strand during DNA replication.<sup>10</sup> Base-pairing rules permit maintenance of reciprocal methylation during subsequent cycles of DNA replication, which, in turn, offers a mechanism for passing a nongenetic trait from cell to cell. In this way, DNA methylation is considered a long-term, stable epigenetic trait.

Although demethylation is known to be an essential process that occurs during certain stages of development, the mechanism of DNA demethylation is less understood than that of methylation. Demethylation may play an essential role in modulating brain plasticity or transcriptional responses to hormones, and targeted or global loss of methylation has been associated with cancer, cardiovascular disease, and other pathologies. Global decreases in DNA methylation can be caused by suppression of DNMT1 methyl transferase. Recent evidence suggests that demethylation may occur by other enzymatic mechanisms that rely on excision-repair mechanisms to replace methyl cytosine with cytosine following deamination. These mechanisms may promote global or targeted changes in methylation. One possible pathway for demethylation involves oxidation of methyl cytosines to hydroxymethyl cytosine by the ten-eleven translocation (Tet) enzymes. Growing evidence suggests that hydroxymethyl cytosine formation is an intermediate stage in DNA demethylation possibly due to its increased susceptibility to deamination.<sup>11</sup> At this time, it is unclear whether 5 hydroxymethyl cytosine may have other essential functions as an epigenetic marker capable of regulating gene expression and chromatin structure; however, the function of the Tet proteins appears to be important for replication-independent DNA demethylation.<sup>12</sup>

### Histone modification

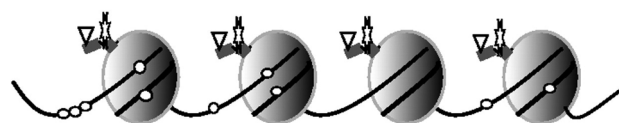
Histones undergo a variety of posttranslational modifications, including acetylation, methylation, phosphorylation, and ubiquitination, that lead to changes in chromatin structure with consequences for gene expression.<sup>13</sup> The “histone code” hypothesis suggests that different types and combinations of modifications differentially alter chromatin structure and transcriptional potential.<sup>14</sup> Acetylation of the  $\epsilon$ -amino group of lysine residues in the aminoterminal

tails of histones H3 and H4 is the best-characterized histone modification most consistently demonstrated to promote transcription. Histone acetylation is catalyzed by histone acetyl transferases, which are recruited to acetylation sites via requisite transcriptional cofactors, such as cyclic AMP response element-binding protein and p300.<sup>15</sup> Histone deacetylation is associated with CpG methylation and inactive chromatin structure. Four classes of HDACs catalyze deacetylation, and they are themselves regulated by posttranslational modification.<sup>16,17</sup> Histone lysine methylation represents another important class of histone modification that alters gene expression, although the determinants of expression (repression or promotion) are complex; they depend on the position of the lysine and the extent of its methylation and are not fully elucidated as yet. Similar to histone acetylation, histone methylation is readily reversible, with many known histone lysine methyltransferases and demethylases that target specific lysines and specific mono-, di-, or trimethylation states.<sup>18-20</sup> Figure 1 depicts the relationships between DNA methylation and common histone modifications in epigenetic regulation of gene expression.

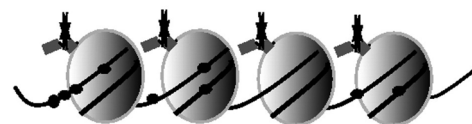
### Noncoding RNA

Long noncoding RNAs can silence genes, owing, in part, to their recruitment of remodeling complexes, such as the polycomb complex, that promote histone methylation. These RNAs can also recruit RNA-binding proteins that impair histone deacetylation or that inhibit transcription factor binding to promoter regions.<sup>21</sup> Through these and

### Active Chromatin



### Inactive Chromatin










- |   |              |   |                             |
|---|--------------|---|-----------------------------|
|  | nucleosome   |  | Acetyl-lysine               |
|  | histone tail |  | Methyl-lysine--activating   |
|  | CpG          |  | Methyl-lysine--inactivating |
|  | Methyl CpG   |   |                             |

Figure 1. Chromatin structure and epigenetic tags. Nucleosomes are indicated as spheres, with sites of methylation and acetylation on DNA and histone proteins listed in the key (reprinted with permission from reference 1).

other mechanisms, long noncoding RNAs are essential for imprinting and X-chromosome inactivation and play key roles in cardiac development.<sup>22</sup> Small inhibitory RNAs and dicer-dependent microRNAs, as short noncoding RNAs, have also been shown to play a role in transcriptional suppression through several mechanisms, including the recruitment of specific argonaute proteins to form epigenetic remodeling complexes that promote histone deacetylation, histone methylation, and DNA methylation.<sup>23,24</sup> The protein interaction world-interacting RNAs (21–30 nt) are a single stranded subclass of these small noncoding RNAs that have been shown to play a role in maintaining the transgenerational inheritance of RNA-induced epigenetic silencing.<sup>25</sup>

### RNA epigenetics

Posttranscriptional RNA modifications represent another type of epigenetics, RNA epigenetics.<sup>26</sup> In particular, RNA (tRNA, mRNA, and rRNA) can undergo methylation at a variety of positions (Fig. 2) in the nucleotide base, as well as at the 2' position of the ribose, and these methylation events can modulate function.<sup>27</sup> The RNA methyltransferases comprise four families and utilize S-adenosylmethionine as a universal methyl donor. In addition, there is growing evidence for RNA demethylases that may modulate gene expression. RNA methylation has different functional conse-

quences, including stabilization, enhanced function, and quality control. In tRNA, for example, modifications are found in certain regions of the tRNA and can contribute to tertiary structure and the accuracy of tRNA recognition. This field of RNA epigenetics is in its infancy but promises to offer yet another level of complex epigenetic regulation of gene expression.

### EPIGENETIC CHANGES AND CARDIOVASCULAR DISEASE

There is much discussion of late regarding so-called missing heritability in complex cardiovascular diseases, such as hypertension and atherosclerosis. Some investigators have posited that epigenetic changes may account for some of this missing heritability. Sequence variation can, for example, create or eliminate CpG sites that are methylation targets and, as a result, contribute to a change in phenotype. For example, expression of the NDSUFB6 respiratory chain protein is decreased in type 2 diabetes mellitus, and a single nucleotide polymorphism (SNP) in the promoter region of this gene creates a CpG site with methylation-dependent suppression of gene expression demonstrated in patient muscle biopsy samples.<sup>28</sup> In addition, nutrition and environmental exposures during critical periods in life, such as in utero<sup>29</sup> or during periods of famine,<sup>30</sup> can alter epigenetically the expression of genes

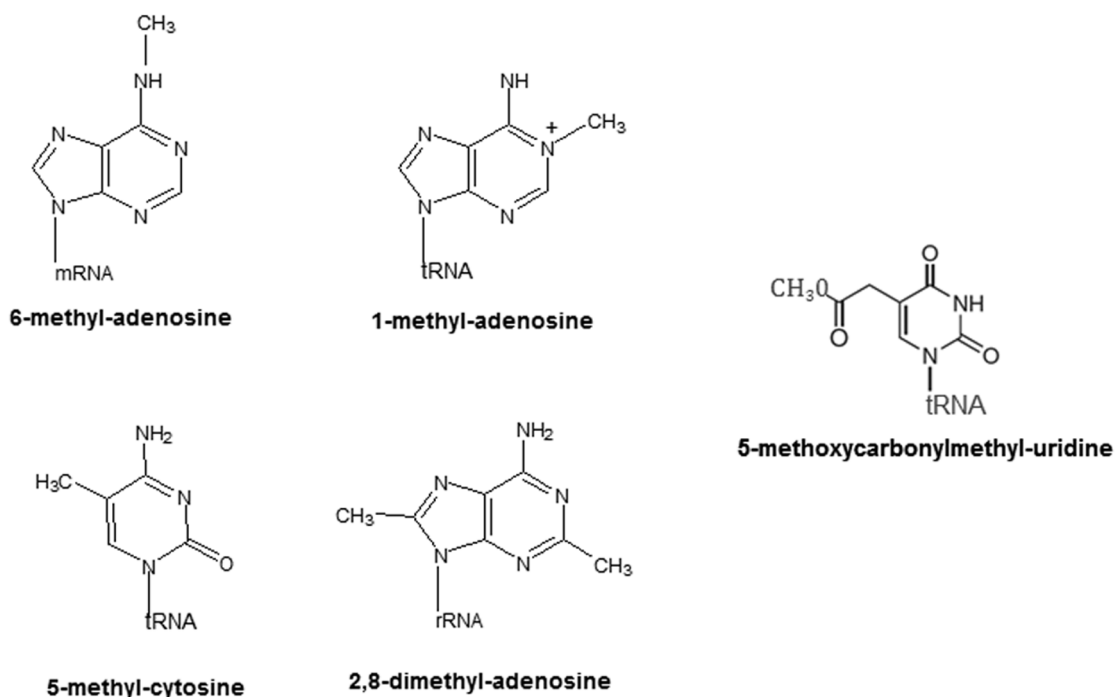


Figure 2. RNA methylation products. Common methylated bases found in mRNA, tRNA, and rRNA.

that contribute to disease risk later in life, such as atherosclerosis or hypertension. In part, this outcome may be a consequence of dietary deficiency of folate or vitamin B12 or of choline (a betaine precursor necessary for folate-independent methylation of homocysteine), essential for methylation reactions that may epigenetically govern gene expression.

One of the known risk factors for atherosclerosis and vascular disease, homocysteine, may exert its actions via an epigenetic mechanism also involving methylation reactions (Fig. 3). Homocysteine is a key determinant of the methylation cycle, in that it is methylated to methionine (in a folate- and vitamin B12-dependent fashion), which undergoes S-adenosylation to form S-adenosylmethionine (SAM), which is the principal methyl donor for all methylation reactions in cells. The specificity of methylation depends on the substrate specificity of any one of the more than 100 methyltransferases in mammalian cells. Furthermore, SAM is converted to S-adenosylhomocysteine (SAH) with methylation. The SAM-to-SAH ratio defines the methylation potential of a cell, and hyperhomocysteinemic states decrease this ratio, leading to decreased methylation potential. Several lines of evidence support the view that homocysteine can lead to global DNA hypomethylation<sup>31,32</sup> and can also suppress transcription of *cyclin A* in endothelial cells. This gene-specific effect is exerted through demethylation of a CpG site in the core promoter, eliminating the binding of methyl CpG-binding protein 2, which, in turn, limits HDAC binding and increases accumulation of acetylated H3 and H4 histones to suppress gene expression.<sup>33</sup> Thus, although DNA hypomethylation and histone acetylation are associated with transcriptional permissive chromatin, the open conformation of chromatin

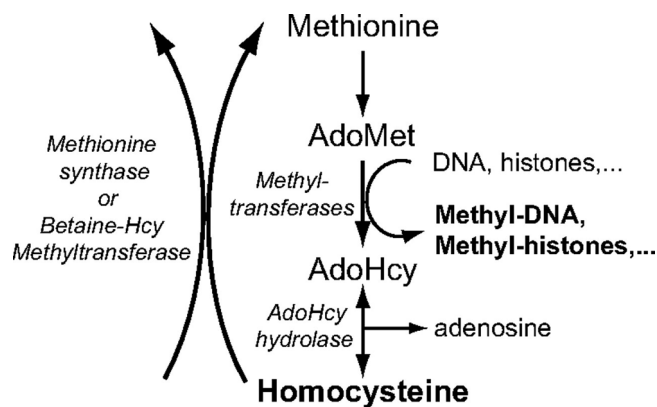


Figure 3. Methylation determinants and homocysteine metabolism. AdoMet: S-adenosylmethionine; AdoHcy: S-adenosylhomocysteine (reprinted with permission from reference 1).

may also allow for augmented access by repressor proteins, leading to transcriptional suppression. Similar epigenetic regulatory mechanisms have been reported to account for changes in *apoA-1* and *apoA-IV* in hyperhomocysteinemia.<sup>34</sup> In contrast, other genes are upregulated by homocysteine-induced DNA hypomethylation of their promoters. One example is the homocysteine-induced increase in p66shc expression in endothelial cells, which correlates with promoter hypomethylation and contributes to oxidant stress.<sup>35</sup>

Epigenetic regulation of cardiovascular development<sup>36</sup> and cardiovascular stem cell biology<sup>37</sup> may be linked to the cardiovascular disease propensity, as well. Epigenetic regulation controls vascular smooth muscle phenotype in health and disease.<sup>38</sup> Archer and colleagues<sup>39</sup> have also shown that epigenetic attenuation of mitochondrial superoxide dismutase (*SOD2*) expression leads to pulmonary artery smooth muscle cell proliferation and resistance to apoptosis through a redox-dependent mechanism.<sup>40</sup> Recent work suggests that increased HDAC activity contributes to the vascular pathobiology of pulmonary arterial hypertension and that inhibition of histone deacetylation is effective in attenuating disease progression and in reducing established disease in a rat model of hypoxia-induced pulmonary hypertension.<sup>41</sup> These studies tested a class I HDAC inhibitor and a broader-spectrum drug that inhibits class I, II, and IV HDAC, each of which reduced pulmonary artery pressure, right ventricular hypertrophy, and vascular muscularization to similar extents, with overlapping effects on histone acetylation and target gene expression. Similarly, other studies explored the ability of other select HDAC inhibitors to preserve right ventricular function and pulmonary blood flow in hypoxia-induced pulmonary hypertension in rats, reporting that class I HDAC inhibitors improved right ventricular function by lessening hypertrophy and reducing apoptotic and inflammatory signaling.<sup>42</sup> In contrast, in a pulmonary artery banding model of right ventricular pressure overload, use of broad-spectrum HDAC inhibitors had a detrimental effect on right ventricular function, promoting dysfunction and hypertrophy, possibly due to antiangiogenic effects of these treatments.<sup>43</sup> This latter study reinforces the concept that targeted HDAC inhibitors may lead to more beneficial treatment strategies and that a better understanding of the underlying causative mechanisms for right heart dysfunction is paramount. Similar approaches have been used to regulate responses to heart failure in order to mitigate remodeling responsible for left ventricular dysfunction. Various studies have shown a role for HDAC in promoting maladaptive remodeling<sup>44</sup> and in HDAC inhib-

itors as promising agents to lessen heart failure in rat models of cardiomyopathy.<sup>45</sup>

Epigenetic regulation by miRNAs may also contribute to cardiovascular disease processes. There is a growing list of miRNAs that modulate cellular responses to hypoxia and/or coordinately regulate pulmonary vascular changes involved in the development of pulmonary arterial hypertension.<sup>46,47</sup> For example, bioinformatics analysis suggested that miR-21 may coordinately modify many targets involved in the pathogenic pathways that contribute to pulmonary hypertension.<sup>48</sup> In vivo, analysis shows that this miRNA is upregulated in tissues from rodent models and humans with pulmonary hypertension. In vivo, inhibition of miR-21 was found to lessen right ventricular systolic pressure and pulmonary artery muscularization in a mouse model of pulmonary hypertension but failed to reduce hypoxia-induced right ventricular hypertrophy, whereas inhibition of miR-17 had a greater effect on heart and lung function in the hypoxia and monocrotaline models of pulmonary hypertension.<sup>49</sup> Similarly, other studies implicate upregulation of miR-145 in smooth muscle proliferation during hypoxia-induced pulmonary artery hypertension, as inhibition of miR-145 significantly lessened vascular remodeling and right ventricular dysfunction in the hypoxia model of pulmonary artery hypertension.<sup>50</sup> This study also reported that miR-145 is upregulated in lungs of patients with idiopathic and heritable forms of pulmonary artery hypertension, suggesting its potential importance in human disease pathology. Other miRNAs, including those that have protective actions, have similarly been shown to regulate the vascular remodeling that is characteristic of this disease. Thus, downregulation of miR-204 in pulmonary hypertension was found to correlate with disease severity and smooth muscle cell proliferation, and delivery of synthetic miR-204 was capable of mitigating disease in an animal model of pulmonary hypertension.<sup>51</sup> Similarly, restoration of miR-424 and miR-503 expression attenuated pulmonary hypertension in an animal model, by mechanisms that may include their ability to regulate endothelial modulation of smooth muscle cell proliferation.<sup>52</sup> Overall, these and other studies not mentioned here suggest a growing importance of miRNAs as critical regulators of complex cardiovascular phenotypes and possible targets for therapeutic intervention.

## CONCLUSIONS

Epigenetic regulation appears to be essential for cardiovascular development and differentiation. In addition, aberrant epigenetic mechanisms, brought about by environmental determinants (homocysteine, vitamin deficiency

states) or on a variant genetic background (SNPs that alter DNA methylation potential), can contribute to disease pathogenesis and offer potential novel therapeutic targets.

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