Epigenetic Modification in Coronary Atherosclerosis

JACC Review Topic of the Week

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ABSTRACT

Coronary artery disease (CAD) and its major complication, acute myocardial infarction (AMI), are the leading causes of disability and death worldwide. An individual’s risk of developing CAD and MI is modulated by an interplay between genetic and lifestyle factors. It is now clear that epigenetics may play a central role in the development of CAD because epigenetic patterns are affected by the environment and can modulate gene expression. Here, the authors discuss the major epigenetic changes that contribute to CAD and the latest discoveries on the influence of the environment on epigenetic profiles in the development of CAD. (J Am Coll Cardiol 2019;74:1352–65) © 2019 by the American College of Cardiology Foundation.

The term epigenetics defines heritable and temporary changes in gene expression and function carried out by genomic mechanisms (deoxyribonucleic acid [DNA] methylation, histone modifications, and ribonucleic acid [RNA]-based mechanisms) that leave the DNA sequence unchanged. The importance of epigenetics lies in its strong dependence on environmental factors that can alter the epigenome and modulate gene expression.

The role of epigenetics in the pathophysiology of coronary atherosclerosis is getting much attention and has led to the belief that investigation of epigenetics in coronary artery disease (CAD) development is pivotal for a full and clear understanding of the disease. Epigenetic modifications greatly contribute to coronary atherosclerosis and are sensitive to environmental risk factors linked to CAD (1). During the development of atherosclerotic plaque, extensive epigenetic changes occur in the biology of endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and macrophages, and in inflammation, cholesterol metabolism, and homocysteine homeostasis (2).

Different experimental approaches have led to growing data on the epigenetic contribution to CAD, allowing for exploration of the entire epigenetic profile of cells and tissues. Here, we discuss the latest discoveries on epigenetic modifications that contribute to the pathogenesis of CAD and how epigenetic modifications respond to environmental changes (Central Illustration).

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HIGHLIGHTS
- Epigenetic modifications that contribute to coronary atherosclerosis are links between genetics and the environment in CAD development.
- Results of studies conducted thus far have been rather inconsistent.
- These inconsistencies in the epigenetic field highlight the need for further research.

HISTONE MODIFICATIONS
Histone proteins package the DNA into nucleosomes. A number of post-transcriptional modifications to the N-terminal histone tail of nucleosomes regulate the chromatin state and modulate accessibility of the DNA to key proteins involved in gene transcription. Among these modifications, histone acetylation and methylation have been widely investigated in atherosclerosis (3). Numerous enzymes can modify histones by adding or removing modifications. Among them, alterations of the expression level of Class II histone deacetylases (HDACs), which remove acetyl groups on the histones, have been linked to atherosclerosis. HDAC2 can be down-regulated by oxidized low-density lipoprotein (ox-LDL), resulting in increased oxidative stress (4).

HDAC3 seems to have a protective role for endothelial integrity, because in physiological conditions, increased expression of HDAC3 has been observed in areas prone to develop atherosclerosis. Indeed, deletion of HDAC3 was linked to reduced EC survival and enhanced atherosclerosis (5). In advanced human plaques, increased HDAC9 was associated with MMP1 and MMP2 expression in proinflammatory macrophages (6). Deficiency of HDAC9 was linked to increased levels of the ATP-binding cassette transporter A1, ATP-binding cassette subfamily G member 1 (ABCA1), and peroxisome proliferator activated receptor gamma, thus preventing cholesterol efflux through up-regulation of histone H3 and H4 acetylation (7).

Alterations in histone modifications have been linked to cardiovascular function. The proximal promoter of the NOS3 gene, which encodes the endothelial nitric oxide synthase (eNOS or eNOS3), is characterized by a specific profile of histone modifications, and changes in this profile are associated with activation or repression of eNOS in response to environmental stimuli (8). Methylation of histone H3K4 has been correlated with stage-specific progression of atherosclerosis (9), whereas a global increase in trimethylation of H3K27 has been observed in late-stage atherosclerotic plaques (10).

The close interaction between histones and DNA makes these proteins of primary importance in many DNA-dependent regulatory processes and therefore important players in disease development and progression.

DNA METHYLATION MODIFICATIONS
DNA methylation, a highly conserved epigenetic modification, refers to covalent binding of a methyl group to the cytosine base of the 5′-CpG-3′ dinucleotide, known as 5-methylcytosine, affecting genome stability, gene expression, and development (11). Key alterations in DNA methylation pertain to methylase and demethylase enzymes (12).

GLOBAL DNA METHYLATION. Several DNA methylation patterns have been described in patients with CAD; however, studies conducted thus far have had major variations in their results (Table 1). These variations are probably due to the heterogeneity of cell types in the atherosclerotic plaque and to incomplete penetrance of genetic/epigenetic alterations. Furthermore, differences in cellular composition in diseased and healthy tissues may relate to the differences in methylation profiles. Emerging single-cell sequencing technologies could, in the future, overcome these discrepancies.

GENE-SPECIFIC DNA METHYLATION. Another approach to the study of methylation changes is assessment of the methylation status of specific genes. The results of some recent studies are shown in Table 2. Changes in the methylation status of “target” genes may affect functional pathways involved in atherogenesis (Figure 1).

ESTROGEN RECEPTORS AND ATEROGENESIS. The effects of estrogens are mediated mainly by estrogen receptor α (ERα) and estrogen receptor β (ERβ). An altered ERα/ERβ ratio has been linked to the development of metabolic diseases (13). ERα and ERβ are considered atheroprotective because they can regulate the biology of ECs and VSMCs, and epigenetic changes affecting their expression characterize vascular aging and atherosclerosis. Several investigators have observed inactivation through methylation of the gene encoding ERα in vascular tissues. ERα and ERβ have been shown to be hypermethylated during in vitro senescing of ECs and
VSMCs derived from human coronary atherosclerotic tissues and plaque regions of the ascending aorta (14).

OXIDATIVE STRESS. eNOS generation in ECs is believed to be essential for a healthy cardiovascular system. The chromatin structure at the NOS3 gene promoter is transcriptionally permissive in ECs and repressive in non-ECs. In physiological conditions, ECs show hypomethylation of the eNOS3 promoter, which results in expression of the gene; accordingly, cell types that physiologically do not express eNOS, such as VSMCs, show hypermethylation of the gene promoter (15). In pathological conditions, such as atherosclerosis, ECs show a low expression level of NOS3 messenger, whereas the expression of NOS2 and NOS1 is up-regulated in several cell types, such as VSMCs, that usually do not express NOS genes (16).

HOMOCYSTEINE METABOLISM. Studies show that global DNA hypermethylation observed in CAD is accentuated by hyperhomocysteinemia that represents a risk factor for CAD, because it correlates with decreased production of NO, VEGF, and protein kinase B (Akt), resulting in disruption of angiogenesis, VSMC proliferation, oxidative stress, and EC damage (17). However, data in this regard are conflicting (18,19).

LIPOPROTEIN METABOLISM. Ox-LDLs are present in atherosclerotic plaque in high concentration compared with circulating levels (20). ECs treated
with ox-LDL show enhanced global DNA methylation. Interestingly, upon repeated exposure to ox-LDL, ECs display resistance to apoptosis due to an epigenetic reprogramming apparently related to hypomethylation of promoter regions of proapoptotic genes and hypermethylation of antiapoptotic genes (21). Ox-LDL exerts its atherogenic effects upon binding with its main receptor on ECs, LOX-1, encoded by the OLR1 gene (22,23). Indeed, the epigenetic effect of ox-LDL is mostly mediated by LOX-1, as treatment of ECs with LOX-1 antibody attenuates this effect (21).

| Gene | DNA Methylation | Gene Expression | Study Design | Method | First Author, Year (Ref. #)
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<tbody>
<tr>
<td>ERα</td>
<td>Hypermethylation of ERα promoter</td>
<td>Not measured</td>
<td>Human coronary atherosclerotic plaques and normal proximal aorta</td>
<td>Southern blot analysis</td>
<td>Post et al., 1999 (14)</td>
</tr>
<tr>
<td>ERβ</td>
<td>Hypermethylation of ERβ promoter</td>
<td>Down-regulated</td>
<td>Plaque and plaque-free regions of human vascular tissues</td>
<td>MS-PCR and combined bisulfite restriction analysis</td>
<td>Kim et al., 2007 (73)</td>
</tr>
<tr>
<td>eNOS3</td>
<td>Hypomethylation of eNOS3 promoter</td>
<td>Up-regulated</td>
<td>ECs in physiological conditions</td>
<td>Bisulfite sequencing</td>
<td>Chan et al., 2004 (75)</td>
</tr>
<tr>
<td>eNOS3</td>
<td>Hypermethylation of eNOS3 promoter</td>
<td>Down-regulated</td>
<td>VSMCs in physiological conditions</td>
<td></td>
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</tr>
<tr>
<td>DDAH2</td>
<td>Hypermethylation of DDAH2 promoter</td>
<td>Down-regulated</td>
<td>EPCs of patients with CAD (n = 25) and healthy subjects (n = 15)</td>
<td>Bisulfite sequencing</td>
<td>Niu et al., 2014 (17)</td>
</tr>
<tr>
<td>PDGF</td>
<td>Hypermethylation of PDGF</td>
<td>Up-regulated</td>
<td>ECs</td>
<td>MS-PCR</td>
<td>Zhang et al., 2012 (18)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Hypermethylation of MCP-1 promoter</td>
<td>Up-regulated</td>
<td>Peripheral blood of ApoE−/− mice (n = 36) and control mice (n = 12)</td>
<td>Nested MS-PCR</td>
<td>Wang et al., 2013 (74)</td>
</tr>
<tr>
<td>p66shc</td>
<td>Hypermethylation of p66shc promoter</td>
<td>Up-regulated</td>
<td>Human ECs treated with LDL</td>
<td>MS-PCR</td>
<td>Kim et al., 2012 (75)</td>
</tr>
<tr>
<td>KLF2</td>
<td>Hypermethylation of KLF2 promoter</td>
<td>Down-regulated</td>
<td>Human ECs</td>
<td>DNMT activity assay</td>
<td>Kumar et al., 2013 (76)</td>
</tr>
<tr>
<td>LDLR</td>
<td>Hypomethylation of LDLR promoter</td>
<td>Up-regulated</td>
<td>Blood of patients with FH (n = 9B)</td>
<td>Bisulfite DNA treatment and pyrosequencing</td>
<td>Guay et al., 2013 (77)</td>
</tr>
<tr>
<td>LOX-1, ANXAS, BAX, CASP8</td>
<td>Hypomethylation of gene promoters</td>
<td>Up-regulated</td>
<td>Human ECs treated with ox-LDLs</td>
<td>Gene-specific promoter methylation analysis</td>
<td>Mitra et al., 2011 (21)</td>
</tr>
<tr>
<td>BCL2, cIAP-1</td>
<td>Hypermethylation of gene promoters</td>
<td>Down-regulated</td>
<td>Human ECs</td>
<td>Gene-specific promoter methylation analysis</td>
<td>Mitra et al., 2011 (21)</td>
</tr>
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<td>ABCA1</td>
<td>Hypomethylation of ABCA1 promoter</td>
<td>Not measured</td>
<td>Blood from patients with CAD (n = 3B) and control subjects (n = 50)</td>
<td>Bisulfite DNA treatment and pyrosequencing</td>
<td>Guay et al., 2014 (78)</td>
</tr>
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<td>ABCA1</td>
<td>TIMP1</td>
<td>Hypermethylation</td>
<td>Down-regulated</td>
<td>Peripheral blood of patients with atherosclerosis (n = 150) and healthy control subjects (n = 150)</td>
<td>NT MS-PCR</td>
</tr>
<tr>
<td>ACAT1</td>
<td>Hypomethylation</td>
<td>Up-regulated</td>
<td>Human ECs treated with ox-LDLs</td>
<td>Gene-specific promoter methylation analysis</td>
<td>Mitra et al., 2011 (21)</td>
</tr>
<tr>
<td>FOX3</td>
<td>Hypermethylation of FOX3 promoter</td>
<td>Down-regulated</td>
<td>Peripheral blood of patients with ACS (n = 18B) and control subjects (n = 68B)</td>
<td>MS-PCR</td>
<td>Ja et al., 2013 (24)</td>
</tr>
<tr>
<td>IL-6</td>
<td>Hypermethylation of IL-6 promoter</td>
<td>Not measured</td>
<td>Peripheral blood of patients with CAD (n = 212) and control subjects (n = 218)</td>
<td>Bisulfite DNA treatment and pyrosequencing</td>
<td>Zuo et al., 2016 (26)</td>
</tr>
</tbody>
</table>

ABCA1 = ATP-binding cassette transporter A1; ACAT1 = acyl coenzyme A acyltransferase 1; ANXAS = annexin A5; ApoE = apolipoprotein E; BAX = BCL2 associated X; BCL2 = B-cell lymphoma 2; CAD = coronary artery disease; CASP3 = caspase 3; cIAP1 = cellular inhibitor of apoptosis protein 1; DDAH2 = dimethylarginine dimethylaminohydrolase 2; DMTN = DNA methyltransferase 2; eNOS3 = endothelial nitric oxygen synthase 3; ERα = estrogen receptor α; ERβ = estrogen receptor β; FD4 = fatty change; FOX3 = forkhead box P3; FOX3 = forkhead box P3; GI6 = intercellulin-6; KLF2 = Krüppel-like factor 2; LDLR = low-density lipoprotein receptor; LOX-1 = lectin-like oxidized low-density lipoprotein receptor-1; MCP-1 = monocyte chemoattractant protein-1; MS-PCR = methylation-specific polymerase chain reaction; NT MS-PCR = nested touchdown methylation-specific polymerase chain reaction; ox-LDL = oxidized low-density lipoprotein; PDGF = platelet-derived growth factor; TIMP1 = TIMP metalloproteinase inhibitor 1.
Changes in the methylation status of "target" genes may affect functional pathways involved in atherogenesis, including oxidative stress (A), homocysteine metabolism (B), LDL metabolism (C), and inflammation (D). Blue up arrows indicate increased methylation or expression level of the genes; blue down arrows indicate decreased methylation or expression level of the genes. Akt = protein kinase B; ANXA5 = annexin A5; BAX = BCL2 associated X; BCL2 = B-cell lymphoma 2; CAD = coronary artery disease; CASP3 = caspase-3; cIAP-1 = cellular inhibitor of apoptosis protein 1; EC = endothelial cell; eNOS3 = endothelial nitric oxide synthase 3; FOXP3 = forkhead box P3; IL-6 = interleukin-6; INF = interferon; KLF2 = Krüppel-like factor 2; LDL = low-density lipoprotein; LOX-1 = lectin-like oxidized low-density lipoprotein receptor-1; MCP-1 = monocyte chemoattractant protein-1; NO = nitric oxide; NOS1/2 = nitric oxide synthase genes; ox-LDL = oxidized low-density lipoprotein; PDGF = platelet-derived endothelial growth factor; SMC = smooth muscle cell; VEGF = vascular endothelial growth factor; VSMC = vascular smooth muscle cell.
FIGURE 1 Continued

C. LDL Metabolism

**Physiological Condition**

- LDL
- Ox-LDL

**Pathological Condition**

- LDL
- Ox-LDL

**Hypomethylation**

- p66shc gene expression
- Mediating endothelial oxidative stress and fatty streak formation

**Hypermethylation**

- KLF2
- Impaired endothelial vascular homeostasis

**Hypomethylation**

- Pro-apoptotic genes expression (LOX-1, ANXA5, BAX and CASP3)

**Hypermethylation**

- Anti-apoptotic genes expression (BCL2 and cIAP-1)

D. Inflammation

**Physiological Condition**

- Leukocyte from controls
- Higher methylation level of IL-6 promoter
- Lower IL-6 expression

**Pathological Condition**

- Leukocyte from CAD patients
- Lower methylation level of IL-6 promoter
- Higher IL-6 expression

**Maintenance of Physiological Status**

**Atherosclerotic Plaque Development**

**Regulation of inflammatory gene expression (INF-γ, IL-6, PDGF and FOXP3) via DNA methylation**
miRNAs involved in the regulation of pivotal steps leading to development of atherosclerotic plaque and disease progression, such as cholesterol and lipid homeostasis (A), EC dysfunction (B), VSMC activation (C), and inflammation (D). ICAM = intercellular adhesion molecule; LDLR = low-density lipoprotein receptor; miRNA = microRNA; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; VCAM = vascular cell adhesion molecule; other abbreviations as in Figure 1.

Continued on the next page
INFLAMMATION. The expression of proinflammatory genes, such as interferon gamma (INF-γ), interleukin 6 (IL-6), forkhead box P3 (FOXP3), platelet-derived growth factor (PDGF), and intercellular adhesion molecule 1 (ICAM-1), appears to be regulated through DNA methylation (24,25).

The association of IL-6 promoter methylation status with cardiovascular disease (CVD) risk was investigated in leukocytes of patients with CAD (including those with acute myocardial infarction [AMI]). The study showed significantly lower methylation levels in patients with CAD compared with control subjects, suggesting an inverse association between methylation and risk for CAD, particularly AMI (26).

Given the dynamic nature and tissue heterogeneity of atherosclerosis, defining the precise role of DNA methylation in the pathogenesis of this condition has been challenging.

RNA-BASED MECHANISMS

Based on data from the Encyclopedia of DNA Elements consortium, >70% of the human genome is known to be transcribed into RNA; however, only ~2% codes for proteins (27).

The noncoding ribonucleic acids (ncRNAs) can be divided into constitutively expressed transcripts and regulatory ncRNAs. Regulatory ncRNAs are active in the regulation of the chromatin state and expression of other RNAs and are usually subdivided according to their length into short (e.g., microRNAs) or long noncoding ribonucleic acids (lncRNAs) (28).

Microribonucleic acids (miRNAs) are capable of modulating gene expression at the transcriptional and post-transcriptional levels and have emerged as key players in the pathophysiology of the cardiovascular system and, importantly, in the pathogenesis of atherosclerosis (29).

The role of IncRNAs in the epigenetic control of gene expression has recently been recognized. To date, the functions of only a few hundred IncRNAs have been characterized and the understanding of their role in CVD is very limited (30).

CELLULAR miRNAs AND CAD. Numerous cellular miRNAs regulate important alterations that occur in ECs, VSMCs, and macrophages, resulting in imbalanced lipid homeostasis and cholesterol accumulation, EC dysfunction, VSMC proliferation, and inflammation (Figure 2). The most relevant miRNAs are listed in Table 3; however, this list keeps expanding as novel miRNAs and their role in atherogenesis are identified.

CHOLESTEROL AND LIPID HOMEOSTASIS. The low-density lipoprotein receptor (LDLR) is an important target gene for miRNAs. Recently, miR-144A has been identified as a negative regulator of LDLR expression.
and activity. Indeed, miR-148a inhibition was shown to increase the clearance of circulating low-density lipoprotein (LDL), resulting in reduced plasma LDL cholesterol levels in mice (31).

**OLR1**, encoding LOX-1 (22,23), is centrally implicated in a series of key processes involved in atherosclerosis, from plaque development to its rupture (32,33). We recently identified a binding site for miR-24 in the 3'-untranslated region of **OLR1** that is "naturally" mutated by a single-nucleotide polymorphism (SNP), rs1050286 (G/A). miR-24 inhibits the expression of **OLR1** and this effect is influenced by rs1050286 SNP, indicating that this SNP may modify LOX-1 susceptibility to atherosclerosis (34).

### ENDOTHELIAL DYSFUNCTION
miR-181b and miR-146a have been identified as cytokine-responsive miRNAs with an atheroprotective role in the regulation of NF-κB signaling. miR-181b expression is reduced in plasma of subjects with angiographically

### TABLE 3 Cellular miRNAs in Coronary Atherosclerosis

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Functional Role</th>
<th>Source</th>
<th>Samples</th>
<th>First Author, Year (Ref. #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-223</td>
<td>Repression of genes implicated in cholesterol biosynthesis (HMGCST, SC5M10) and HDL uptake (SRBI)</td>
<td>Liver</td>
<td>Mouse</td>
<td>Vickers et al., 2014 (80)</td>
</tr>
<tr>
<td>miR-27b</td>
<td>Repression of genes involved in lipid metabolism (PPARG, GPAM, ANGPTL1, and NOD7)</td>
<td>Hepatocyte cells (Huh7)</td>
<td>Human</td>
<td>Vickers et al., 2013 (81)</td>
</tr>
<tr>
<td>miR-148a</td>
<td>Negative regulator of LDLR expression and activity</td>
<td>Human hepatocyte cell lines (Huh7, HepG2)</td>
<td>Mouse/hepatocyte cell (HEPA)</td>
<td>Goedeke et al., 2015 (31)</td>
</tr>
<tr>
<td>miR-24</td>
<td>miR-24 targets 3'-UTR of OLR1 in presence of G allele of rs1050286 SNP</td>
<td>HepL1 (A/G rs1050286 SNP), HepG2 (A/A rs1050286 SNP)</td>
<td>Human</td>
<td>Morini et al., 2016 (34)</td>
</tr>
<tr>
<td>miR-33 family</td>
<td>miR-33a and -b target ABC1A and regulate, in concert with the SREBP host genes, cholesterol homeostasis</td>
<td>Human THP1 Mø and HepG2</td>
<td>Mouse/hepatocyte cell (HEPA)</td>
<td>Rayner et al., 2010 (82)</td>
</tr>
<tr>
<td>miR-17-3p</td>
<td>TNFα-induced miR-17-3p and miR-31 operate a feedback control on the expression of TNFα-induced adhesion molecules ICAM-1 and E-selectin, respectively</td>
<td>HUVECs</td>
<td>Human</td>
<td>Suárez et al., 2010 (83)</td>
</tr>
<tr>
<td>miR-146a</td>
<td>The kinase-responsive miR-146a is active in the inhibition of NF-κB signaling by targeting the TNF receptor-associated factor 6 and the interleukin-1 receptor-associated kinase-1 in ECs and macrophages. miR-146a is involved in the repression of EC adhesion molecule expression.</td>
<td>HUVECs Wild-type and miR-146a-/- mouse intimal cells</td>
<td>Human/mouse</td>
<td>Li et al., 2015 (36)</td>
</tr>
<tr>
<td>miR-126</td>
<td>miR-126 targets the 3’-UTR of VCAM-1 thus limiting leukocyte adhesion. miR-126-5p targets DLK-1, reducing atherosclerotic lesions formation</td>
<td>HUVECs Mouse aorta</td>
<td>Human/mouse</td>
<td>Schober et al., 2014 (84)</td>
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<tr>
<td>miR-92a</td>
<td>miR-92a targets the 3’-UTR of KLK2, mediating ECs flow response. Ox-LDL-induced miR-92a expression promotes endothelial activation and development of atherosclerotic lesions</td>
<td>HUVECs LDLr/-/- mice</td>
<td>Human</td>
<td>Loyer et al., 2014 (85)</td>
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<tr>
<td>miR-143</td>
<td>Depletion of miR-143 and miR-145 reduced expression and function of SMC contractile proteins in VSMCs</td>
<td>miR-143-/- and miR-145 deficient mice</td>
<td>Mouse</td>
<td>Cordes et al., 2009 (39)</td>
</tr>
</tbody>
</table>

**ABCGL** = ATP-binding cassette subfamily G member 1; **AMPKα1** = AMP-activated protein kinase alpha catalytic subunit 1; **ANGPTL3** = angiopoietin-like 3; **CPT1a** = carnitine palmitoyltransferase 1A; **DLK-1** = delta-like noncanonical Notch ligand 1; **EC** = endothelial cell; **GPAM** = glycerol-3-phosphate acyltransferase, mitochondrial; **HDL** = high-density lipoprotein; **HMGCST** = 3-hydroxy-3-methylglutaryl-CoA synthase 1; **HUVEC** = human umbilical endothelial cell; **ICAM-1** = intercellular adhesion molecule 1; **Mø** = macrophage; **NOD7** = N-deacetylase and N-sulfotransferase 1; **NF-κB** = nuclear factor kappa-light-chain-enhancer of activated B cells; **OLR1** = oxidized low-density lipoprotein receptor 1; **PPARG** = peroxisome proliferator activated receptor gamma; **SCAM10** = sterol-C4-methyl oxidase-like; **SNP** = single-nucleotide polymorphism; **SRBI** = scavenger receptor class B type 1; **SREBP** = sterol regulatory element-binding protein; **TNFα** = tumor necrosis factor; **UTR** = untranslated regions; **VCAM-1** = vascular cell adhesion molecule 1; **VSMC** = vascular smooth muscle cell; other abbreviations as in Table 2.
FOAM CELLS. A large number of miRNAs have been identified as playing a role in foam cell formation through inhibition of macrophage cholesterol efflux via ABCA1 (29).

Many miRNAs regulate the balance between proatherosclerotic M1 and antiatherosclerotic M2 phenotypes. Among these miRNAs, miR-33 appears to have a pivotal role in the promotion of the M1 phenotype (44).

**CIRCULATING miRNAs AND CAD.** An important aspect of miRNA biology is their remarkable stability in the bloodstream, where they can be easily detected. Because of this feature, circulating miRNAs may be used as potential biomarkers for diagnosis of different stages of CAD (Table 4). Many investigators have studied the expression levels of circulating miRNAs in the plasma of patients with stable or unstable CAD (45–47); however, no miRNA alone or combination of miRNAs could correctly discriminate between these patients. Numerous studies have been performed to identify a specific and characteristic miRNA profile in the setting of MI. We recently identified different miR-423-5p expression levels in patients with stable CAD compared with those in patients with AMI and suggested that miR-423-5p may be used as an epigenetic biomarker for identification of patients with CAD at risk of developing AMI (48). Although many studies have been performed to identify miRNAs with diagnostic and prognostic values, there is not a common agreement on this

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Regulation</th>
<th>Study Design</th>
<th>Source</th>
<th>Sample</th>
<th>Method</th>
<th>First Author, Year (Ref. #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-126, -17, -92a, -415, -155</td>
<td>Down-regulated</td>
<td>Case-control</td>
<td>Human plasma</td>
<td>Stable CAD (n = 36), control subjects (n = 17)</td>
<td>Array</td>
<td>Fichtlscherer et al., 2011 (45)</td>
</tr>
<tr>
<td>miR-133, -208a</td>
<td>Up-regulated</td>
<td></td>
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<tr>
<td>miR-155</td>
<td>Down-regulated</td>
<td>Case-control</td>
<td>Human PBMCs and plasma</td>
<td>Patients with CAD (n = 56), control subjects (n = 54)</td>
<td>qRT-PCR</td>
<td>Zhu et al., 2014 (46)</td>
</tr>
<tr>
<td>miR-1, -122, -126, -133a/-133b, -199a, -485-5p, -433</td>
<td>Lower expression in patients with 2 or 3 diseased vessels compared with patients with 0 or 1 diseased vessel</td>
<td>Case-control</td>
<td>Human plasma</td>
<td>Patients with UA (n = 19), patients with SA patients (n = 34), control subjects (n = 20)</td>
<td>qRT-PCR</td>
<td>D’Alessandra et al., 2013 (85)</td>
</tr>
<tr>
<td>miR-208</td>
<td>Up-regulated</td>
<td>Case-control</td>
<td>Human plasma</td>
<td>Rats treated with a subcutaneous injection of isoproterenol (n = 8)</td>
<td>qRT-PCR</td>
<td>Ji et al., 2009 (87)</td>
</tr>
<tr>
<td>miR-208a</td>
<td>Up-regulated</td>
<td>Case-control</td>
<td>Human plasma</td>
<td>Patients with CAD (n = 16), patients with AMI (n = 33), control subjects (n = 30)</td>
<td>qRT-PCR</td>
<td>Wang et al., 2011 (47)</td>
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<tr>
<td>miR-423-5p</td>
<td>Plasmin: down-regulated in AMI, TO compared with CAD and up-regulated in AMI, TO</td>
<td>Comparison between stable and unstable CAD patients</td>
<td>Human plasma and PBMCs</td>
<td>Patients with CAD (n = 61), patients with AMI (n = 38) recruited at the time of MI event (AMI, TO) and after 6 months (AMI, TI)</td>
<td>qRT-PCR</td>
<td>Rizzacasa et al., 2019 (48)</td>
</tr>
</tbody>
</table>

**TABLE 4** Circulating miRNAs in Coronary Atherosclerosis

Proven CAD (35). miR-146a targets TRAF6 and IRAK1 in ECs and macrophages, resulting in decreased atherosclerotic plaques in humans and mice and suggesting that it may be involved in limiting inflammatory signaling in these cells (36).

miR-126 is one of the most richly expressed miRNAs in ECs and has been linked to the flow-dependent regulation of inflammation and angiogenesis (37). Similarly, miR-92a is highly expressed in ECs and dynamically regulated by shear stress both in vitro and in vivo, and the exposure of ECs to disturbed flow increases its expression (38).

**VSMC ACTIVATION.** The phenotypic switching of VSMCs during atherogenesis to proliferate, migrate, and secrete extracellular matrix proteins and cytokines is of fundamental importance. In basal conditions, miR-143 and miR-145 are among the most highly expressed miRNAs in VSMCs (39). Conversely, their expression is reduced in injured and atherosclerotic regions (40). miR-143/-145 target important genes involved in VSMC activation (41) and are important regulators of VSMC contractile function (42).

Our laboratory showed that miRNA has-let-7g regulates autophagy and apoptosis in ox-LDL-treated VSMCs (43).

**INFLAMMATION.** Monocytes that differentiate into macrophages exert a crucial role in atherogenesis. Once in the plaque, macrophages become lipid-laden foam cells. A large number of miRNAs have been...
theme, and further studies are needed to establish usefulness of miRNAs for risk stratification of patients with CAD.

**IncRNAs AND CVD.** The cellular IncRNA profile is altered in several pathological states, including CVD. The study of IncRNAs in CAD and their potential use as epibiomarkers or therapeutic targets is expanding, and several IncRNAs have been characterized and linked to CAD (Table 5).

The expression of Inc-H19 in human atherosclerosis was the first suggestion of involvement of IncRNAs in CVD (49). Recently, elevated plasma levels of H19 were reported in the blood of CAD patients (50). Previous studies proved that a tight interaction between IncRNAs, miRNAs, and genes is fundamental for fine-tuning of several biological processes; for example, the Inc-RP5-833A20.1/miR-382-5p/NFIA pathway has been demonstrated to be essential for the regulation of cholesterol homeostasis (51).

To date, the vast majority of IncRNAs involved in atherosclerosis have been studied in cardiac tissues and ECs. Among them, particular attention has been addressed to myocardial infarction-associated transcript 1 (MI) miRNA expression (52–54), antisense noncoding RNA in the INK4 locus (ANRIL) (55–57), and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (58–60). Expression levels of these IncRNAs have been studied in atherosclerotic and

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**TABLE 5 LncRNAs in Coronary Atherosclerosis**

<table>
<thead>
<tr>
<th>LncRNA</th>
<th>Functional Role</th>
<th>Source</th>
<th>Samples</th>
<th>First Author, Year (Ref. #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H19</td>
<td>Up-regulated in patients with atherosclerosis (n = 30) compared with healthy control subjects (n = 30). H19 knockdown reduced lipid accumulation and relieved inflammation in these cells. H19 regulates adipogenesis and inflammation response in ox-LDL-treated macrophages by up-regulating miR-130B.</td>
<td>Serum</td>
<td>Human</td>
<td>Bitarafan et al., 2019 (49)</td>
</tr>
<tr>
<td>RPS-883A20.1</td>
<td>LncRNA-RP5-833A20.1 decreases NFIA expression, which, by inducing miR-382-5p expression, regulates cholesterol transport across the cell membrane via boosting the expression of ABCA1 and ABCG1. Lnc-RP5-833A20.1/miR-382-5p/NFIA pathway is essential for regulation of cholesterol homeostasis and inflammatory reactions.</td>
<td>ox-LDL-treated Raw264.7 macrophages</td>
<td>Mouse</td>
<td></td>
</tr>
<tr>
<td>MIAT</td>
<td>MIAT locus presents 6 SNPs significantly linked to a higher risk of MI. MIAT levels are useful to distinguish patients with STEMI from those with NSTEMI. MIAT acts as a ceRNA, targeting miR-150-5p to regulate EC function.</td>
<td>Human acute monocytic leukemia macrophage-derived foam cells</td>
<td>Human</td>
<td>Hu et al., 2015 (50)</td>
</tr>
<tr>
<td>ANRIL</td>
<td>The 9p21.3 risk allele variant is linked to CAD and promotes atherosclerosis by regulating ANRIL expression, which, in turn, modifies the activity of 2 nearby cyclin-dependent kinase inhibitors that are involved in regulating the cell cycle and cellular proliferation.</td>
<td>Human subject homozygous for the risk allele (n = 63) and homozygous for the reference allele (n = 61). Patients with CAD homozygous for the risk allele (n = 21) and homozygous for the reference allele (n = 21). The linear form of ANRIL is positively associated with risk of atherosclerosis, increased cell proliferation, and decreased apoptosis. The circular form of ANRIL confers atheroprotection in VSMCs and macrophages, induc- ing nuclear stress and activation of p53, resulting in induction of apoptosis and inhibition of proliferation.</td>
<td>PBMCs from healthy subjects and patients with different degrees of CAD</td>
<td>Human Jarinova et al., 2009 (54)</td>
</tr>
<tr>
<td>MALAT1</td>
<td>MALAT1, interacting with β-catenin, promotes transcription of CD36, a scavenger receptor involved in foam cell formation. MALAT1 acts as a sponge for miR-22-3p and protects the endothelium from ox-LDL-induced endothelial dysfunction. Inhibition of MALAT1 impairs vascularization and EC proliferation in vitro.</td>
<td>THP1-derived macrophages</td>
<td>Human</td>
<td>Huangfu et al., 2018 (57)</td>
</tr>
<tr>
<td>H19</td>
<td>Knockdown of CHROME reduces cholesterol efflux and formation of HDL particles in hepatocytes. CHROME specifically targets miR-27b, miR-33a, miR-33b, and miR-128 that repress cholesterol efflux.</td>
<td>HUVECs</td>
<td>Human</td>
<td>Tang et al., 2015 (58)</td>
</tr>
<tr>
<td>CHROME</td>
<td>CHROME is highly expressed in plasma and atherosclerotic plaques from subjects with coronary artery disease. Knockdown of CHROME reduces cholesterol efflux and formation of HDL particles in hepatocytes.</td>
<td>Plasma and atherosclerotic plaques from patients with symptomatic and asymptomatic carotid stenosis compared with healthy control subjects</td>
<td>Human</td>
<td>Hennessy et al., 2019 (61)</td>
</tr>
</tbody>
</table>

**ANRIL** = antisense noncoding RNA in the INK4 locus; **ceRNA** = competing endogenous RNA; **CHROME** = cholesterol homeostasis regulator of miRNA expression; **MALAT1** = metastasis-associated lung adenocarcinoma transcript 1; **MI** = myocardial infarction; **MIAT** = myocardial infarction-associated transcript; **NFIA** = nuclear factor IA; **VEGF** = vascular endothelial growth factor; other abbreviations as in Tables 2 to 4.
nonatherosclerotic arteries obtained from the same patients. ANRIL and MIAT were found to be up-regulated, whereas MALAT1 was down-regulated in atherosclerotic regions compared with non-atherosclerotic regions (60).

Recently, a novel lncRNA called CHROME (cholesterol homeostasis regulator of miRNA expression) has been identified as highly expressed in plasma and coronary plaques and characterized for its role in the regulation of cholesterol homeostasis (61).

**EPIGENETIC ALTERATIONS IN RESPONSE TO ENVIRONMENTAL CHANGES.** Environmental distress has been shown to perturb biological development with differential sensitivity according to a tissue-specific critical window of susceptibility. The interaction between genes and exogenous stimuli, known as developmental plasticity (62), suggests that organisms attempt to fine-tune the genome response to generate phenotypic profiles fitting the changing environment. Epigenetic inheritance postulates that some epigenetic marks can pass through generations. The in utero developmental period represents a critical time during which the environment can strongly influence the epigenetic “signature,” thus altering gene expression. Antenatal environmental factors have also been linked to altered fetal growth and to permanent biological and physiological changes in the offspring, a process called transgenerational epigenetic inheritance (63). Moreover, during in utero development, the plasticity of tissues is particularly sensitive to environmental factors such as diet, pathological conditions (such as diabetes and hypertension) (64), smoking, chemical contaminants, and social stress (65). Thus, it appears that epigenetic processes are involved in the development of changes in gene expression and play a critical role in mediating fetal programming of adult chronic disease (Central Illustration).

Smoking is one of the major risk factors for atherosclerosis. A meta-analysis of genome-wide DNA methylation changes associated with cigarette smoking highlighted thousands of differentially methylated CpGs. A comparison between current and former smokers versus never-smokers revealed that alterations in CpG methylation pattern persist many years after smoking cessation (66).

**EPIGENETIC THERAPY AND CVD**

The modifiable nature of epigenetic marks makes them key targets for future therapies. Recent studies suggest that these alterations can be reversed using pharmaceutical agents and nutraceuticals. Those that have been particularly studied with possible therapeutic applications in inflammation and CAD are DNA methyltransferase inhibitors (DNMTis), histone acetyltransferase inhibitors, histone deacetylase inhibitors, histone methylation inhibitors, and bromodomain and extra-terminal motif (BET) inhibitors (67). In addition to synthetic DNMTis, there also are natural DNMTis available in food. These inhibitors have been extensively reviewed elsewhere (68).

**CONCLUSIONS**

The epigenome dynamically responds to changes in the environment during a person’s lifetime, participating in the control of fundamental biological processes and representing an important link among life experiences, phenotypic expression, and disease risk. Undoubtedly, it seems that limiting human exposure to causative events through strategies targeting specific environmental risk factors (improved nutrition, reduced exposure to pollutants, and healthy lifestyles) in adulthood and particularly during early developmental stages represents the most cost-effective opportunity to tackle CAD incidence with results over the short-, medium-, and long-term.

Epigenetics has dramatically influenced our understanding of gene regulation in atherosclerotic CAD. Further work will provide an opportunity to develop strategies for prevention, early diagnosis, and accurate personalized therapies. However, it must be recognized that the results of epigenetic studies thus far have been inconsistent due to the heterogeneity of analyzed tissues and activity of the disease process, differences in the demographics of the patients studied, and variations in therapies. Furthermore, multiple abnormalities in the epigenome may coexist and regulate each other, and these interactions have not been fully explained. Nevertheless, these interactions highlight the need for future rigorous studies to understand the role of epigenetics in the evolution of CAD, its diagnosis, its prognosis, and the development of potential therapeutics.

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