THE PRESENT AND FUTURE

JACC REVIEW TOPIC OF THE WEEK

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. In fact, 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).
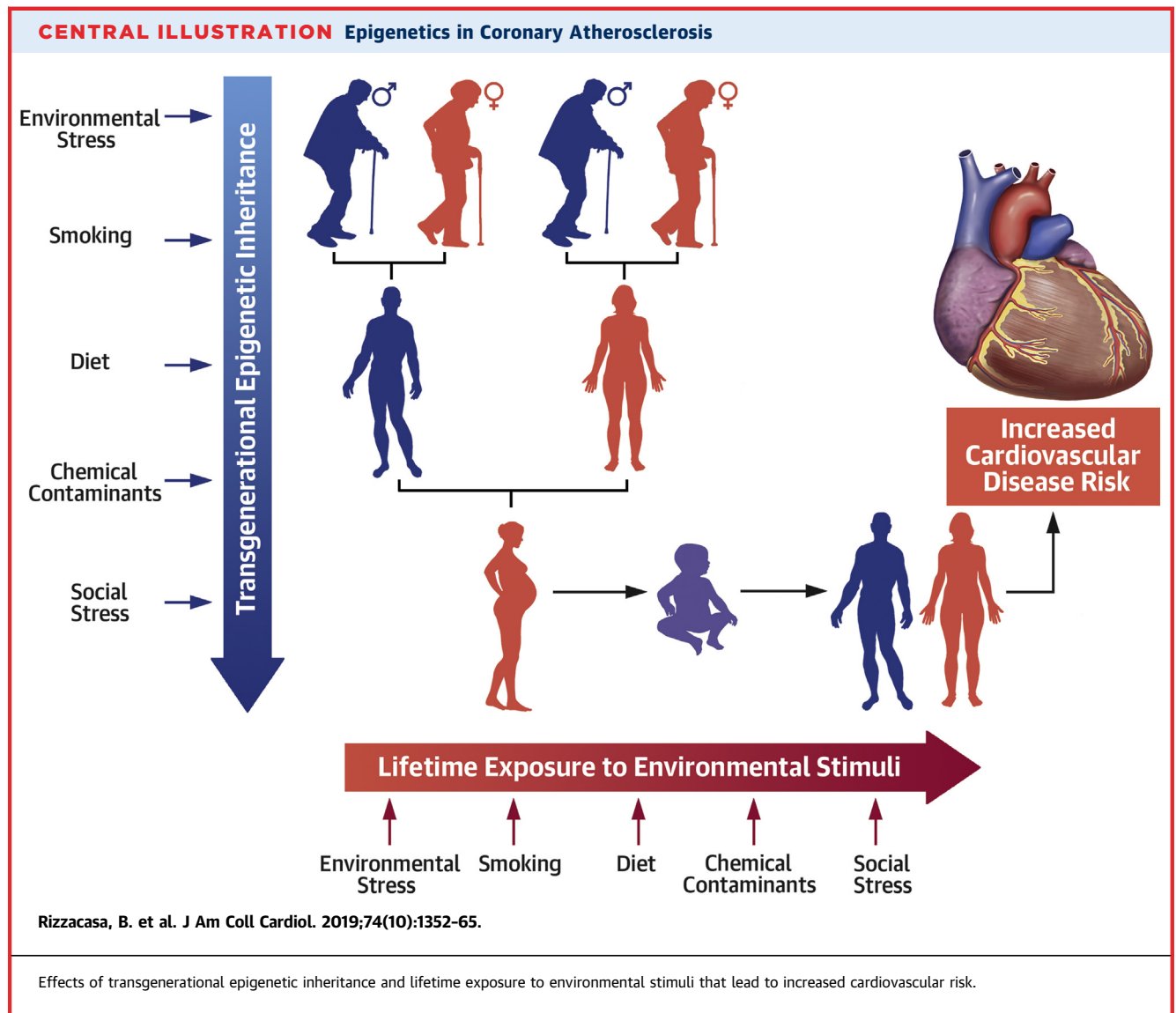
GLOBAL DNA METHYLATION. Several DNA methylation patterns have been described in patients with CAD; however, studies conducted thus far have had major variations in their results (**Table 1**). These variations are probably due to the heterogeneity of cell types in the atherosclerotic plaque and to incomplete 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 ATHEROGENESIS. 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

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

CAD = coronary artery disease

CVD = cardiovascular disease

EC = endothelial cell

lncRNA = long noncoding ribonucleic acid

miRNA = microribonucleic acid

ncRNA = noncoding ribonucleic acid

ox-LDL = oxidized low-density lipoprotein

SNP = single-nucleotide polymorphism

VSMC = vascular smooth muscle cell

CENTRAL ILLUSTRATION Epigenetics in Coronary Atherosclerosis

Rizzacasa, B. et al. J Am Coll Cardiol. 2019;74(10):1352–65.

Effects of transgenerational epigenetic inheritance and lifetime exposure to environmental stimuli that lead to increased cardiovascular risk.

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

TABLE 1 Global DNA Methylation in CVD

Global DNA Methylation Status	Study Design	Method	First Author, Year (Ref. #)
Hypomethylation	Human arterial samples: control subjects (n = 3), fatty streaks (n = 23), advanced lesions (n = 29)	HPLC and bisulfite sequencing	Hiltunen et al., 2002 (69)
Hypomethylation	Human femoral artery atherectomy samples (n = 22), Normal mammary artery samples (n = 9)	Methylcytosine-dependent fragment capture and NGS	Aavik et al., 2015 (70)
Hypermethylation	Postmortem donor-matched atherosclerotic and nonatherosclerotic portions of human aortas (n = 15)	Bisulfite DNA treatment and microarray-based DNA methylation analysis	Valencia-Morales et al., 2015 (71)
Hypermethylation	Postmortem donor-matched atherosclerotic and nonatherosclerotic portions of human aorta (n = 1)	Bisulfite DNA treatment and microarray-based DNA methylation analysis	Zaina et al., 2014 (72)

CAD = coronary artery disease; CVD = cardiovascular disease; HPLC = high-performance liquid chromatography; NGS = next-generation sequencing.

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).

TABLE 2 Gene-Specific DNA Methylation in CVD

Gene	DNA Methylation	Gene Expression	Study Design	Method	First Author, Year (Ref. #)
ERα	Hypermethylation of ERα promoter	Not measured	Human coronary atherosclerotic plaques and normal proximal aorta	Southern blot analysis	Post et al., 1999 (14)
ERβ	Hypermethylation of ERβ promoter	Down-regulated	Plaque and plaque-free regions of human vascular tissues	MS-PCR and combined bisulfite restriction analysis	Kim et al., 2007 (73)
eNOS3	Hypomethylation of eNOS3 promoter	Up-regulated	ECs in physiological conditions	Bisulfite sequencing	Chan et al., 2004 (15)
	Hypermethylation of eNOS3 promoter	Down-regulated	VSMCs in physiological conditions		
DDAH2	Hypermethylation of DDAH2 promoter	Down-regulated	EPCs of patients with CAD (n = 25) and healthy subjects (n = 15)	Bisulfite sequencing	Niu et al., 2014 (17)
PDGF	Hypomethylation of PDGF	Up-regulated	ECs	MS-PCR	Zhang et al., 2012 (18)
MCP-1	Hypomethylation of MCP-1 promoter	Up-regulated	Peripheral blood of ApoE$^{-/-}$ mice (n = 36) and control mice (n = 12)	Nested MS-PCR	Wang et al., 2013 (74)
p66shc	Hypomethylation of p66shc promoter	Up-regulated	Human ECs treated with LDL	MS-PCR	Kim et al., 2012 (75)
KLF2	Hypermethylation of KLF2 promoter	Down-regulated	Human ECs	DNMT activity assay	Kumar et al., 2013 (76)
LDLR	Hypomethylation of LDLR promoter	Up-regulated	Blood of patients with FH (n = 98)	Bisulfite DNA treatment and pyrosequencing	Guay et al., 2013 (77)
LOX-1, ANXA5, BAX, CASP3	Hypomethylation of gene promoters	Up-regulated	Human ECs treated with ox-LDLs	Gene-specific promoter methylation analysis	Mitra et al., 2011 (21)
BCL2 cIAP-1	Hypermethylation of gene promoters	Down-regulated			
ABCA1	Hypermethylation of ABCA1 promoter	Not measured	Blood from patients with CAD (n = 38) and control subjects (n = 50)	Bisulfite DNA treatment and pyrosequencing	Guay et al., 2014 (78)
ABCA1 TIMP1	Hypermethylation	Down-regulated	Peripheral blood of patients with atherosclerosis (n = 150) and healthy control subjects (n = 150)	NT MS-PCR	Ma et al., 2016 (79)
ACAT1	Hypomethylation	Up-regulated			
FOXP3	Hypermethylation of FOXP3 promoter	Down-regulated	Peripheral blood of patients with ACS (n = 188) and control subjects (n = 68)	MS-PCR	Jia et al., 2013 (24)
IL-6	Hypomethylation of IL-6 promoter	Not measured	Peripheral blood of patients with CAD (n = 212) and control subjects (n = 218)	Bisulfite DNA treatment and pyrosequencing	Zuo et al., 2016 (26)

ABCA1 = ATP-binding cassette transporter A1; ACAT1 = acetyl coenzyme A acetyltransferase 1; ANXA5 = annexin A5; ApoE = apolipoprotein E; BAX = BCL2 associated X; BCL2 = B-cell lymphoma 2; CAD = coronary artery disease; CASP3 = caspase 3; cIAP-1= cellular inhibitor of apoptosis protein 1; DDAH2 = dimethylarginine dimethylaminohydrolase 2; DNMT = DNA methyltransferase; eNOS3 = endothelial nitric oxygen synthase 3; EPC = circulating endothelial progenitor cell; ERα =estrogen receptor α; ERβ = estrogen receptor β; FH = familial hypercholesterolemia; FOXP3 = forkhead box P3; IL-6 = interleukin-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 metallopeptidase inhibitor 1.

FIGURE 1 Gene-Specific DNA Methylation in CAD
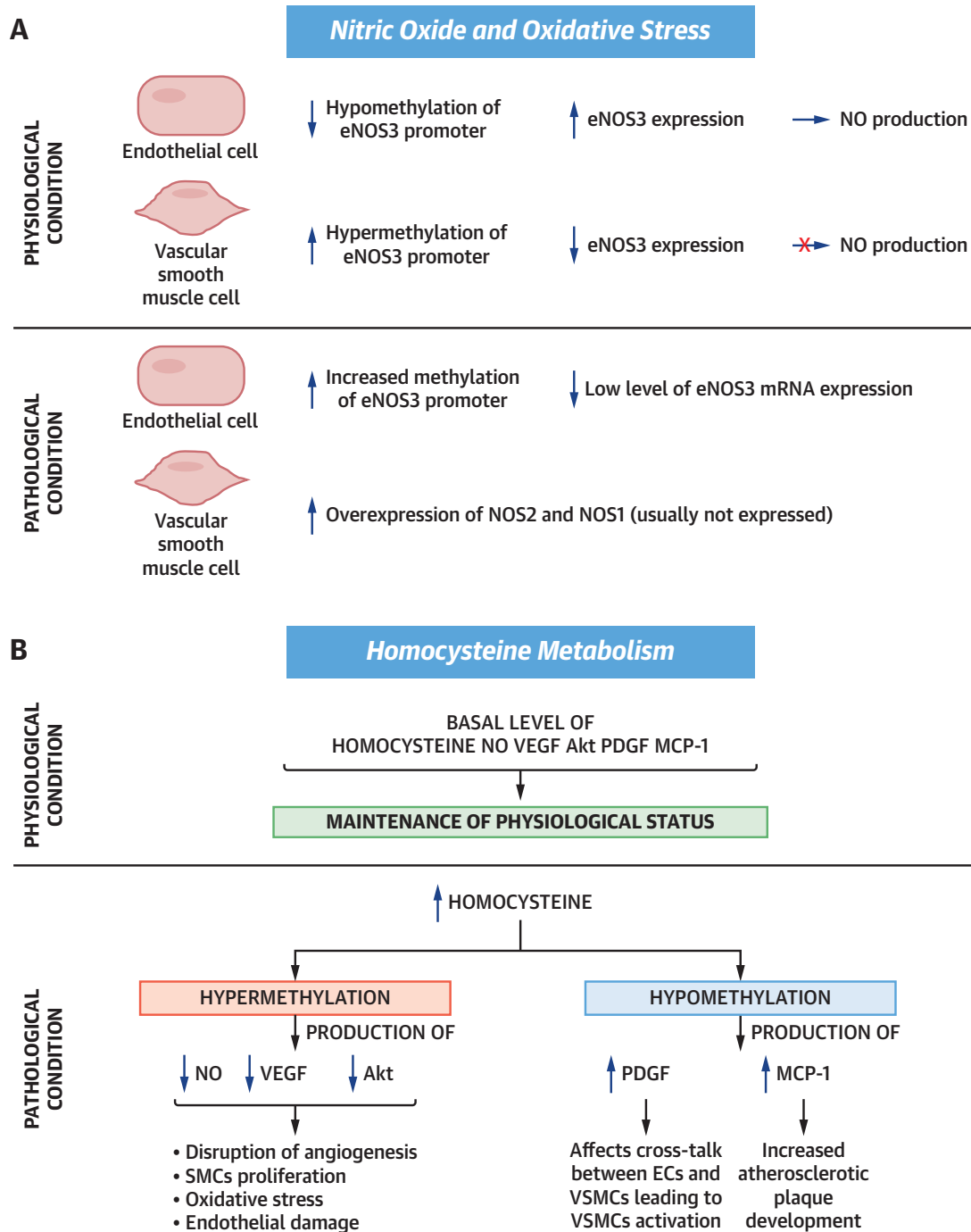
Changes in the methylation status of "target" genes may affect functional pathways involved in atherogenesis, including oxidative stress **(A)**, homocysteine metabolism **(B)**, LDL metabolism **(C)**, and inflammation **(D)**. **Blue up arrows** indicate increased methylation or expression level of 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 P3IL-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.
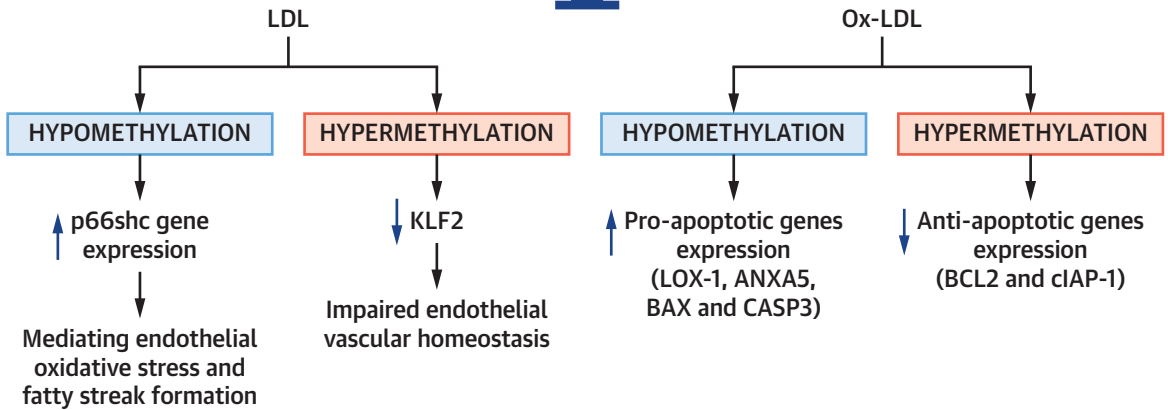
FIGURE 1 Continued

C

PHYSIOLOGICAL CONDITION

LDL Metabolism

LDL   Ox-LDL

UNALTERED GENE METHYLATION STATUS

MAINTENANCE OF PHYSIOLOGICAL LDL HOMEOSTASIS

PATHOLOGICAL CONDITION

LDL   Ox-LDL

LDL — LDL | LDL   Ox-LDL — Ox-LDL

| **HYPOMETHYLATION** | **HYPERMETHYLATION** | **HYPOMETHYLATION** | **HYPERMETHYLATION** |

↑ p66shc gene expression

↓ KLF2

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

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

Mediating endothelial oxidative stress and fatty streak formation

Impaired endothelial vascular homeostasis

D

Inflammation

Regulation of inflammatory gene expression (INF-γ, IL-6, PDGF and FOXP3) via DNA methylation

PHYSIOLOGICAL CONDITION

Leukocyte from controls

↑ Higher methylation level of IL-6 promoter   ↓ Lower IL-6 expression
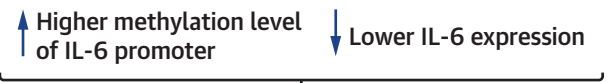
MAINTENANCE OF PHYSIOLOGICAL STATUS

PATHOLOGICAL CONDITION

Leukocyte from CAD patients

↓ Lower methylation level of IL-6 promoter   ↑ Higher IL-6 expression

ATHEROSCLEROTIC PLAQUE DEVELOPMENT

FIGURE 2 MiRNAs in CAD
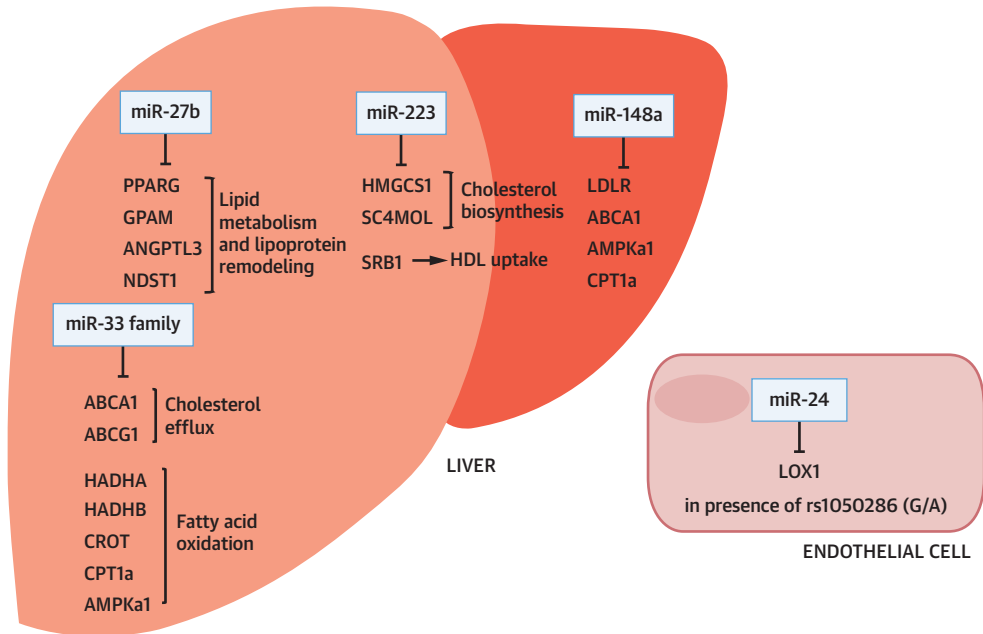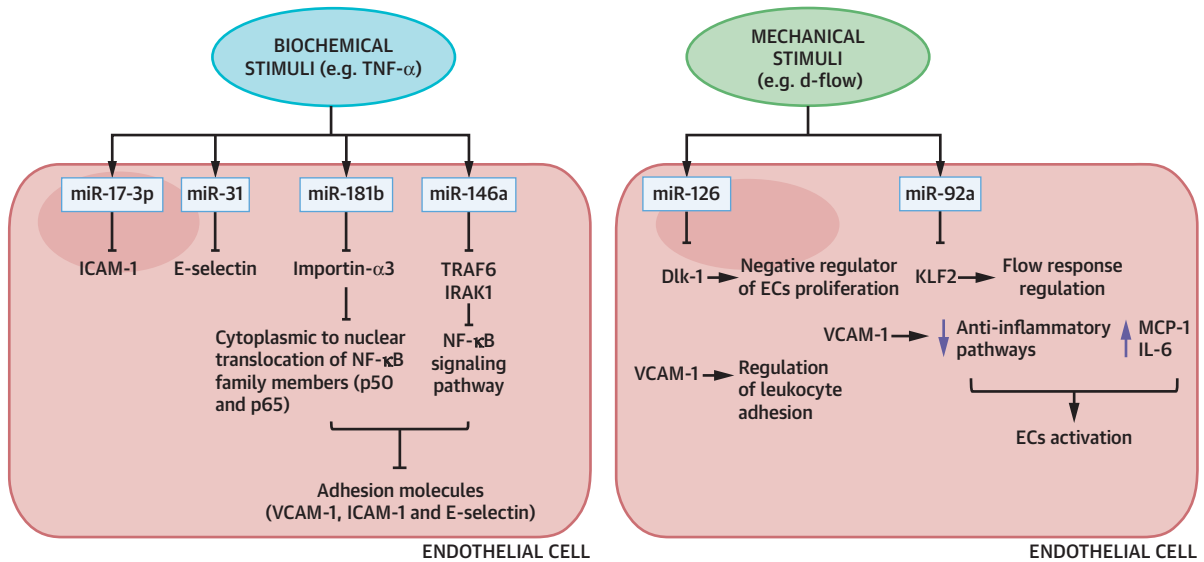
miRNAs involved in the regulation of pivotal steps leading to development of atherosclerotic plaque and disease progression, such as cholesterol and lipid homeostasis **(A)**, EC dysfunction **(B)**, VSMC activation **(C)**, and inflammation **(D)**. ICAM = intercellular adhesion molecule; LDLR = low-density 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.

FIGURE 2   Continued

C   Vascular Smooth Cells Activation
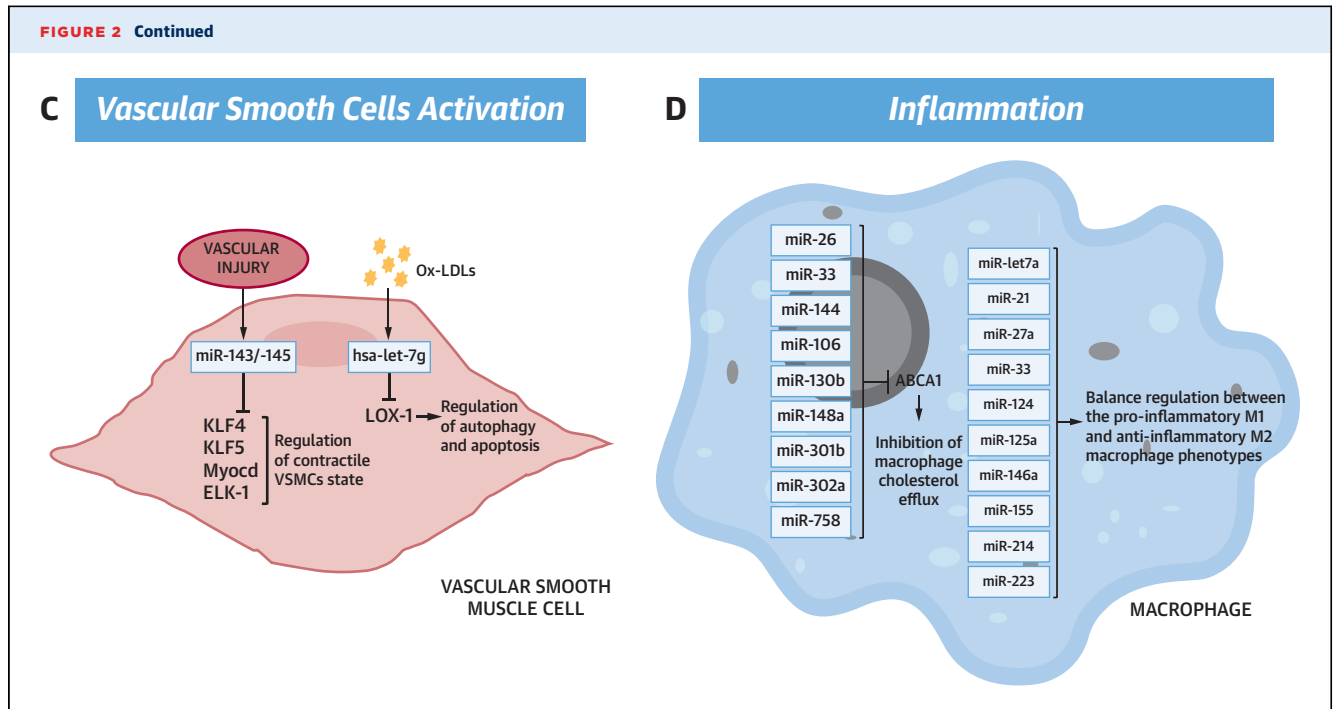
D   Inflammation

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 lncRNAs in the epigenetic control of gene expression has recently been recognized. To date, the functions of only a few hundred lncRNAs 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-148a has been identified as a negative regulator of LDLR expression

TABLE 3   Cellular miRNAs in Coronary Atherosclerosis

miRNA	Functional Role	Source	Samples	First Author, Year (Ref. #)
miR-223	Repression of genes implicated in cholesterol biosynthesis (*HMGCS1*, *SC4MOL*) and HDL uptake (*SRB1*)	Liver	Mouse	Vickers et al., 2014 (80)
miR-27b	Repression of genes involved in lipid metabolism (*PPARG*, *GPAM*, *ANGPTL3*, and *NDST1*)	Hepatocyte cells (Huh7)	Human	Vickers et al., 2013 (81)
miR-148a	Negative regulator of LDLR expression and activity Repression of genes involved in lipid metabolism (*ABCA1*, *AMPKa1*, and *CPT1a*)	Human hepatocyte cell lines (Huh7, HepG2) Mouse hepatocyte cell (HEPA)	Human/mouse	Goedeke et al., 2015 (31)
miR-24	miR-24 targets 3′-UTR of *OLR1* in presence of G allele of rs1050286 SNP	HeLa (A/G rs1050286 SNP), HepG2 (A/A rs1050286 SNP)	Human	Morini et al., 2016 (34)
miR-33 family	miR-33a and -b target ABCA1 and regulate, in concert with the SREBP host genes, cholesterol homeostasis	Human THP1 Mø and HepG2	Human/mouse	Rayner et al., 2010 (82)
	In mouse and human cells, miR-33 inhibits the expression of ABCA1. In mouse macrophages, miR-33 also targets ABCG1, reducing cholesterol efflux to nascent HDL.	Mouse peritoneal Mø and HEPA		
	miR-33 promotes the inflammatory M1 macrophages phenotype Inhibition of miR-33 lead to metabolic changes in the plaque macrophages that consequently acquire a M2 phenotype, leading to suppression of plaque inflammation and tissue repair	Mouse peritoneal Mø	Mouse	Ouimet et al., 2015 (44)
miR-17-3p miR-31	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	HUVECs	Human	Suárez et al., 2010 (83)
miR-181b	miR-181b expression is reduced in the aortic intima of ApoE$^{-/-}$ mice. Systemic delivery of miR-181b inhibited NF-κB activation, NF-κB-responsive proinflammatory gene expression, leukocyte accumulation, and atherosclerotic lesion formation. This effect is mediated by importin-α3, a miR-181b target gene. miR-181b inhibited NF-κB only in the vascular endothelium of atherosclerotic lesions	ApoE$^{-/-}$ mice aorta	Mouse	Sun et al., 2014 (35)
miR-146a	The cytokine-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.	HUVECs Wild-type and miR-146a$^{-/-}$ mouse intimal cells	Human/mouse	Li et al., 2015 (36)
miR-126	miR-126 targets the 3′-UTR of VCAM-1 thus limiting leukocyte adhesion miR-126-5p targets DLK-1, reducing atherosclerotic lesions formation	HUVECs Mouse aorta	Human/mouse	Schober et al., 2014 (84)
miR-92a	miR-92a targets the 3′-UTR of KLF2, mediating ECs flow response Ox-LDL-induced miR-92a expression promotes endothelial activation and development of atherosclerotic lesions	HUVECs LDLr$^{-/-}$ mice	Human Human/mouse	Loyer et al., 2014 (85)
miR-143 miR-145	Depletion of miR-143 and miR-145 reduced expression and function of SMC contractile proteins in VSMCs	miR-143$^-$ miR-145$^-$ deficient mice	Mouse	Cordes et al., 2009 (39)

ABCG1 = ATP-binding cassette subfamily G member 1; AMPKa1 = 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; HMGS1 = 3-hydroxy-3-methylglutaryl-CoA synthase 1; HUVEC = human umbilical endothelial cell; ICAM-1 = intercellular adhesion molecule 1; Mø = macrophage; NDST1 = 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; SC4MOL = sterol-C4-methyl oxidase-like; SNP = single-nucleotide polymorphism; SRB1 = scavenger receptor class B type 1; SREBP = sterol regulatory element-binding protein; TNF = tumor necrosis factor; UTR = untranslated region; VCAM-1 = vascular cell adhesion molecule 1; VSMC = vascular smooth muscle cell; other abbreviations as in Table 2.

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 4 Circulating miRNAs in Coronary Atherosclerosis

miRNA	Regulation	Study Design	Source	Sample	Method	First Author, Year (Ref. #)
miR-126, -17, -92a, -145, -155	Down-regulated	Case-control	Human plasma	Stable CAD (n = 36), control subjects (n = 17)	Array	Fichtlscherer et al., 2011 (45)
miR-133, -208a	Up-regulated					
miR-155	Down-regulated. Lower expression in patients with 2 or 3 diseased vessels compared with patients with 0 or 1 diseased vessel	Case-control	Human PBMCs and plasma	Patients with CAD (n = 56), control subjects (n = 54)	qRT-PCR	Zhu et al., 2014 (46)
miR-1, -122, -126, -133a/-133b, -199a, -485-3p, -433	Up-regulated	Case-control	Human plasma	Patients with UA (n = 19), patients with SA patients (n = 34), control subjects (n = 20)	qRT-PCR	D'Alessandra et al., 2013 (86)
miR-208	Up-regulated	Observational	Plasma	Rats treated with a subcutaneous injection of isoproterenol (n = 8)	qRT-PCR	Ji et al., 2009 (87)
miR-208a	Up-regulated in AMI	Case-control	Human plasma	Patients with CAD (n = 16), patients with AMI (n = 33), control subjects (n = 30)	qRT-PCR	Wang et al., 2011 (47)
miR-423-5p	Plasma: down-regulated in AMI_T0 compared with CAD and up-regulated in AMI_T1 compared with AMI_T0. PBMCs: up-regulated in AMI_T1 compared with AMI_T0	Comparison between stable and unstable CAD patients	Human plasma and PBMCs	Patients with CAD (n = 61), patients with AMI (n = 38) recruited at the time of AMI event (AMI_T0) and after 6 months (AMI_T1)	qRT-PCR	Rizzacasa et al., 2019 (48)

AMI = acute myocardial infarction; CAD = coronary artery disease; NSTEMI = non-ST-segment elevation myocardial infarction; PBMC = peripheral blood mononuclear cell; qRT-PCR = quantitative real-time polymerase chain reaction; SA = stable angina; STEMI = ST-segment elevation myocardial infarction; UA = unstable angina.

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 miR-NAs 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 hsa-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 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 5   LncRNAs in Coronary Atherosclerosis

LncRNA	Functional Role	Source	Samples	First Author, Year (Ref. #)
H19	Up-regulated in patients with atherosclerosis (n = 30) compared with healthy control subjects (n = 30).	Serum	Human	Bitarafan et al., 2019 (49)
	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.	ox-LDL–treated Raw264.7 macrophages	Mouse	
RP5-883A20.1	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 the regulation of cholesterol homeostasis and inflammatory reactions.	Human acute monocytic leukemia macrophage-derived foam cells	Human	Hu et al., 2015 (50)
MIAT	MIAT locus presents 6 SNPs significantly linked to a higher risk of MI	Case-control association study	Human	Ishii et al., 2006 (51)
	MIAT levels are useful to distinguish patients with STEMI from those with NSTEMI.	PBMCs from STEMI (n = 274) and NSTEMI (n = 140) patients	Human	Vausort et al., 2014 (52)
	MIAT acts as a ceRNA, targeting miR-150-5p to regulate EC function.	Endothelial cells (RF/6A)	Monkey	Yan et al., 2015 (53)
ANRIL	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.	Healthy subjects 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)	Human	Jarinova et al., 2009 (54)
	The linear form of ANRIL is positively associated with risk of atherosclerosis, increased cell proliferation, and decreased apoptosis.	PBMCs from healthy subjects and patients with different degrees of CAD	Human	Holdt et al., 2010 (55)
	The circular form of ANRIL confers atheroprotection in VSMCs and macrophages, inducing nucleolar stress and activation of p53, resulting in induction of apoptosis and inhibition of proliferation.	Human cell lines and primary cells	Human	Holdt et al., 2016 (56)
MALAT1	MALAT1, interacting with β-catenin, promotes transcription of CD36, a scavenger receptor involved in foam cell formation.	THP1-derived macrophages	Human	Huangfu et al., 2018 (57)
	MALAT1 acts as sponge for miR-22-3p and protects the endothelium from ox-LDL–induced endothelial dysfunction.	HUVECs	Human	Tang et al., 2015 (58)
	Inhibition of MALAT1 impairs vascularization and EC proliferation in vitro.	HUVECs	Human	Michalik et al., 2014 (59)
CHROME	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. CHROME specifically targets miR-27b, miR-33a, miR-33b, and miR-128 that repress cholesterol efflux.	Plasma and atherosclerotic plaques from patients with symptomatic and asymptomatic carotid stenosis compared with healthy control subjects HepG2 HEK293T HepG2	Human	Hennessy et al., 2019 (61)

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.**

theme, and further studies are needed to establish usefulness of miRNAs for risk stratification of patients with CAD.

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

The expression of lnc-H19 in human atherosclerosis was the first suggestion of involvement of lncRNAs 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

lncRNAs, miRNAs, and genes is fundamental for fine-tuning of several biological processes; for example, the lnc-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 lncRNAs involved in atherosclerosis have been studied in cardiac tissues and ECs. Among them, particular attention has been addressed to myocardial infarction-associated transcript (MIAT) (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 lncRNAs have been studied in atherosclerotic and

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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