Diabetes, Metabolic Syndrome and Obesity

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REVIEW

Recent Updates on Epigenetic-Based Pharmacotherapy for Atherosclerosis

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Abstract: Atherosclerosis is one of the most dominant pathological processes responsible in cardiovascular diseases (CVD) caused by cholesterol accumulation accompanied by inflammation in the arteries which will subsequently lead to further complications, including myocardial infarction and stroke. Although the incidence of atherosclerosis is decreasing in some countries, it is still considered the leading cause of death worldwide. Atherosclerosis is a vascular pathological process that is chronically inflammatory and is characterized by the invasion of inflammatory cells and cytokines. Many reports have unraveled the pivotal roles of epigenetics such as DNA methylation, post-translational histone modifications, and non-coding RNAs (ncRNAs) in atherogenesis, which regulate the expression of numerous genes related to various responsible pathways. Many studies have been conducted to develop new therapeutical approaches based on epigenetic changes for combating atherosclerosis. This review elaborates on recent updates on the development of new atherosclerosis drugs whose mechanism of action is associated with the modulation of DNA methylation, posttranslational histone modifications, and ncRNA-based gene regulation.

Keywords: atherosclerosis, DNA methylation, histone modifications, non-coding RNAs, pharmacotherapy

Introduction

Atherosclerosis, the most common underlying pathological condition in cardiovascular disease (CVD), is characterized by the accumulation of cholesterol and inflammation in major arteries, potentially leading to serious clinical outcomes like myocardial infarction (MI) and stroke.[1] In 2015, almost 17 million fatalities worldwide were attributed to CVD, accounting for 31% of all deaths.[2] This issue is particularly pronounced in many Asian countries, where annual death rates range from 103 to 366 per 100,000 adults.[3] Atherosclerosis develops in complicated steps that involve the buildup of lipoproteins like LDL in blood vessels, inflammation initiation, endothelial cell activation, immune cell adhesion, foam cell formation, and the eventual development of atherosclerotic plaques, some of which can become unstable and prone to rupture, leading to MI.[4–8]

Epigenetics is defined as any chromatin change that alters DNA accessibility or chromatin structure to regulate gene expression which includes DNA methylation, posttranslational histone modification, and non-coding RNA (ncRNA) regulated gene expression.[9] Numerous studies suggested that pro-atherogenic factors like LDL cholesterol and oxidized LDL can permanently remodel the epigenome of innate immune system cells.[10] Although epigenetic changes are heritable, they can still be modified with the use of medication.[11]

Many studies have been conducted to develop new therapeutic approaches based on epigenetic changes for combating atherosclerosis through various molecular pathways, so this review elaborates on recent updates on the development of new atherosclerosis drugs whose mechanism of action is associated with the modulation of DNA methylation, post-translational histone modifications, and ncRNA-based gene regulation.

Received: 6 February 2024
Accepted: 19 April 2024
Published: 29 April 2024

Fundamental Concepts in Epigenetics

Epigenetics is the study of changes in gene expression without changes in DNA sequence, which is related to changes in environmental cues, including lifestyle.[12,13] The first epigenetic mechanism is through changes in DNA methylation levels. The addition of methyl groups to CpG sites on the DNA reduces gene expression.[12] The second mechanism is through posttranslational modification which can take the form of adding acetyl, methyl, or other groups to histones.[14] Epigenetic regulation can also involve ncRNA, namely RNA that is produced from DNA transcription like other RNA but does not undergo translation into protein.[9]

DNA methylation is the earliest and most widely studied epigenetic marker and involves the addition of a methyl group (-CH3) at position 5 of cytosine which binds to guanine via phosphate (CpG) to produce 5-methylcytosine.[15,16] In mammals, most CpG islands are found in promoters, gene areas that play a major role in the initiation of gene transcription.[17] Previous studies have shown that DNA methylation has various functions, such as mammalian embryonic development, and can be involved in the development of cancer and metabolic diseases, such as diabetes mellitus, obesity, atherosclerosis, etc.[18–20]

Histone modifications can increase or decrease gene expression depending on the type and location of the modification because the addition of molecules to histones can cause euchromatin (a loose chromatin structure that makes gene expression easier) or heterochromatin (a very dense and dense chromatin structure that makes gene expression difficult).-[14] H3K9me2, which is the addition of two methyl groups (dimethyl) to lysine 9 on the tail of histone H3, is a histone modification that can reduce gene expression, while H3K4me3 will increase gene expression.[21] Acetylation on histones, for example, lysine 27 of histone H3, will increase gene expression.[21]

The term ncRNA refers to RNA that does not code for proteins but this does not mean that ncRNAs have no function in gene regulation.[22,23] ncRNA consists of microRNA (miRNA) and small nucleolar RNA (snoRNA).[22] miRNA is short RNA consisting of approximately 22 nucleotides derived from hairpin or double-stranded RNA precursors which is involved in post-transcription by inhibiting translation either through pairing with target mRNA at six to eight nucleotides, or through activation of the RNA-induced silencing complex (RISC) which causes degradation of target mRNA.[22,24–28] The snoRNA consists of 60–300 nucleotides and acts by forming site-specific modifications on the target RNA through base-pairing in a short region.[22,29]

Role of Epigenetics in Atherogenesis

DNA methylation has been linked to inflammation in atherosclerosis. For instance, DNA hypermethylation in leukocytes is associated with inflammation in chronic kidney disease contributing to mortality. DNA methylation may also play a role in CVD risk.[30] Abnormal blood flow patterns resulting from irregular shear stress can also trigger DNA methylation, as observed in the KLF4 promoter, further suppressing KLF4 expression. Different DNA methyltransferases (DNMTs) may have distinct roles in atherosclerosis, for example, DNMT1 is linked to monocyte expression and CAD risk, while DNMT3a and DNMT3b are implicated in inflammation and macrophage polarization.[31]

TET enzymes, including TET1, TET2, and TET3, can modulate DNA methylation and also play a pivotal role in atherosclerosis. Increased TET1 expression is associated with DNA hypomethylation in atherosclerotic plaques, while TET2 influences the microstructural changes of vascular smooth muscle cells, EC abnormalities, and macrophage recruitment.[32] TET2 modulation of autophagic processes suggests it could be a promising drug target for atherosclerosis. Additionally, TET3-mediated DNA demethylation is crucial for DNA damage repair and gene expression stability in atherosclerosis, emphasizing the importance of these epigenetic changes in disease development.[32]

Histone modifications, such as histone methylation and acetylation, affect how genes are turned on or off in atherosclerosis. These changes are induced by factors such as high blood sugar and enzymes like SETD7, Suv39h1, and LSD1, and they impact gene activity. Histone acetylation typically activates genes by relaxing the chromatin structure, while histone deacetylases (HDACs) remove acetyl groups and play a role in various cell types involved in atherosclerosis, affecting inflammation and plaque stability. Histone acetyltransferases (HATs) are proatherogenic by increasing nuclear acetylation.[33] Inhibiting HATs can have anti-inflammatory effects, such as enhancing cholesterol removal from macrophages. Histone methylation, a more complex modification, can activate or suppress genes based on

specific residues and the number of methyl groups added. In atherosclerosis, reduced histone H3K27me3 levels are associated with increased vascular smooth muscle cell activity, while H3K4me3 methylation can boost the expression of inflammatory genes in response to oxLDL. A histone demethylase, JMJD3, further influences gene expression during atherosclerosis, responding to inflammation and transcriptional activity in macrophages.[34] Bromodomain and extraterminal domain (BET) proteins have been identified as crucial epigenetic regulators that read the acetylation on histone tails, thereby playing a significant role in gene expression regulation.[35] Previous studies have shown that BET inhibitors (BETi) have a potential to reduce inflammation in both mice and humans, indicating their promise as therapeutic interventions for atherosclerosis.[36]

ncRNAs, including miRNAs, lncRNAs, and circular RNAs (circRNAs), do not code for protein synthesis but instead have important roles as master regulators of gene induction in atherosclerosis. miRNAs, composed of 20–40 nucleotides, take part in post-transcriptional gene modulation and are implicated in processes like inflammation, oxidative stress, and cholesterol balance within atherosclerotic lesions, with distinct roles in vascular smooth muscle cells, endothelial dysfunction, and macrophage function.[37] lncRNAs, exceeding 200 nucleotides, play a pivotal role in atherosclerosis, contributing to endothelial cell injury, abnormal vascular smooth muscle cell microstructural changes, and plaque formation. circRNAs, circular molecules derived from pre-mRNAs, also influence atherosclerosis through their interactions with miRNAs and involvement in endothelial cell abnormalities, changes in vascular smooth muscle cell activities, and macrophage-facilitated injury.[38]

miRNAs play pivotal role in atherosclerosis by regulating smooth muscle cells proliferation, migration, and apoptosis, inflammation, and cholesterol and triglyceride metabolism.[39] Increased miR-126 and miR-145 levels were reported to inhibit smooth muscle cells proliferation and migration.[38] miRNA-374 was reported to play pivotal role in regulating proliferation and migration of vascular smooth muscle cells during atherogenesis.[40] miR-155 was known to regulate inflammation and endothelial cell apoptosis, which are important for atherosclerotic plaque development.[38] miR-33a/b regulates cholesterol and triglyceride metabolism, which are risk factors for atherosclerosis.[38] Understanding the roles of these ncRNAs in atherosclerosis holds promise for potential diagnostic and therapeutic applications.[38]

Updates on Epigenetic-Based Pharmacotherapy for Atherosclerosis

Table 1 details updates on epigenetic-based drugs for atherosclerosis treatment. Some drugs were developed based on the histone modifications targeted, while others were developed for modifying DNA methylation and modulating RNA-based gene expression, as detailed below.

Histone Modification-Based Atherosclerosis Pharmacotherapy

Some histone modification-based drugs for atherosclerosis are classified as histone acetyltransferase (HAT) inhibitors, histone deacetylase (HDAC) inhibitors, and histone methyltransferase (HMT) inhibitors (Table 1). Garcinol and MG149 (anacardic acid) are HAT inhibitors that target p300 HAT and early growth response protein 1 (EGR1) and p300 HAT and cellular p300/CBP-associated factor (PCAF), respectively.[41–43] Garcinol inhibits the induction of EGR1 transcription in smooth muscle cells through IL-1β stimulation and plays an important role in the acetylation of Histone 3 at the EGR-1 promoter, while MG149 inhibits MYST1 and the NF-κB pathway.[41–43]

Vorinostat, valproate, panobinostat, romidepsin, and sodium butyrate are classified as HDAC inhibitors, while chaetocin and GSK126 are grouped as histone methylation inhibitors and they all have different mechanisms of action (Table 1). Vorinostat decreases the levels of TNF-α, IL-1b, IL-6, and IFN-γ and valproate mitigates pro-atherogenic endoplasmic reticulum (ER) stress signaling pathways.[44,45] Chaetocin, an inhibitor of H3K9 methyltransferase SUV39H, inhibits SUV39H and decreases the expression of matrix metalloproteinase 9 (MMP9).[46] Panobinostat decreases high-sensitivity C-reactive protein (hsCRP), soluble CD40 ligand (sCD40L), MMP-9, IL-6 as well as total circulating monocytes, romidepsin decreases monocytic adhesion to arterial EC, sodium butyrate downregulates inflammatory mediators such as TNF-α, IL-6, and HMGB1, while GSK126 inhibits lipid formation in both THP-1 and RAW264.7-derived macrophages.[47–50]

The BET protein inhibitor apabetalone (RVX-208) has been found to counter proinflammatory aortic gene expression in a diet-induced obesity mouse model and in human endothelial cells.[36] Furthermore, apabetalone has been shown to

Table 1 Epigenetic-Based Pharmacotherapy for Atherosclerosis

General target	Drug name	Chemistry/ properties	Type of study/ clinical study status	Population/ subjects	Specific target	Mechanism of action	Main Results	Ref.
Histone modification	Garcinol	HAT inhibitor	In vitro	Primary human aortic smooth muscle cells (HASMCs) and rat aortic smooth muscle cells	p300 HAT and EGR1	Inhibiting the induction of EGR1 transcription in smooth muscle cells through IL-1β modulation and playing a role in the acetylation of Histone 3 at the EGR-1 promoter.	Garcinol can regulate the expression of EGR-1 induced by IL-1β by modulating the phosphorylation and acetylation of histone H3.	[41]
	MG149 (anacardic acid)	HAT inhibitor	In vitro and in vivo	BALB/c mice	P300 HAT and PCAF	Inhibiting MYST1 and the NF-κB pathway	The impact of MG149 extends to the spliceosome network and Sestrins, which play a role in DNA repair.	[42] [43]
	Vorinostat	HDAC inhibitor	In vivo	ApoE−/− mice	Class I and class II HDAC enzymes	Decreasing the levels of TNF-α, IL-1b, IL-6, and IFN-γ	The administration of vorinostat to ApoE −/− mice was found to lead to a substantial reduction in the extent of atherosclerotic lesions and was associated with a decrease in oxidative stress and pro-inflammatory markers.	[44]
	Valproate	Selective HDAC inhibitor	In vivo	Wild-type mouse embryonic fibroblasts and hyperglycemic apoE-deficient mice		Mitigating pro-atherogenic endoplasmic reticulum (ER) stress signaling pathways	Valproate's in vivo anti-atherogenic effects align with its ability to impede GSK-3 and disrupt pro-atherogenic ER stress signaling pathways in vitro.	[45]
	Chaetocin	Methyltransferase inhibitor	In vivo	Vascular smooth muscle cells (VSMCs)	Inhibitor of H3K9 methyltransferase SUV39H	Inhibiting SUV39H and decreasing the expression of MMP9	Chaetocin treatment in vivo resulted in decreased H3K9me3 expression, diminished atherosclerotic plaque formation, and increased plaque stability by decreasing necrotic core area and lipid accumulation and increasing collagen content and contractile VSMC phenotype.	[46]
	Panobinostat	HDAC inhibitor	FDA approved	HIV-infected adults on suppressive antiretroviral therapy	Class I, II, and IV HDAC enzymes	Decreasing hsCRP, sCD40L, MMP-9, IL-6 and total circulating monocytes	Panobinostat lowered the level of CRP, LDL receptor, MCP1, E-Selectin, HMGB1 and several inflammatory markers	[47]
	Romidepsin	HDAC inhibitor	In vitro	Primary human aortic endothelial cell (HAEC)	Class I and class II HDAC enzymes	Decreasing monocytic adhesion to arterial EC	Romidepsin significantly attenuated TNFα-induced VCAM-1 expression on HAEC surface and monocyte adhesion by simultaneously inhibiting HDAC1/2.	[48]
	Sodium butyrate	HDAC inhibitor	In vivo	C57BL/6 mice	Zinc sites of class I and II histone deacetylases (HDACs)	Butyrate induces cyclin D1 and D3, inhibits G1-specific cdk4, cdk6 and cdk2, and increases cdk inhibitors, p15INK4b and p21Cip1 by inhibiting histone acetylation.	Butyrate modulates G1-specific cell cycle proteins which inhibits VSMCs proliferation, an important factor in atherogenesis through chromatin remodeling.	[49]
	GSK126	Histone methylation inhibitors (HTMi)	In vivo *and* in vitro	Apolipoprotein E-deficient mouse and human THP-1 cell	Histone methyltransferase EZH2	Inhibiting lipid accumulation in both THP-1 and RAW264.7-derived macrophages, increasing expression levels of ATP-binding cassette transporter A1 and suppressing vascular cell adhesion molecule 1 in human THP-1 cells.	Pharmacological inhibition of EZH2 by GSK126 markedly reduced lipid transportation and monocyte adhesion during atherogenesis.	[50]

DNA methylation	Decitabine	DNMT Inhibitor	In vivo	Apoe/mice fed with Western diet	LXRα, PPARγ, and COL15A1	Downregulating chemokines in macrophages. Inhibiting methylation on COL15A1 gene.	Decitabine treatment inhibited the expression of genes involved in inflammation and chemotaxis in macrophages and increased COL15A1 mRNA and protein levels in SMC.	[51,52]
	RG108	DNMT Inhibitor	In vitro	Human aortic endothelial cells	DNMT3A	Inhibiting the activity of DNMT3A	RG108 partially rescued KLF4 target genes' expression, including NOS3 and MCP-1, indicating the potential of DNA methylation inhibitors to reverse atherogenic gene expression profiles.	[53]
	Hydralazine	DNMT Inhibitor	In vitro	HL-1 cardiomyocytes	DNMT1	Increasing SERCA2a expression by inhibiting methylation on the promoter.	Hydralazine decreased methylation level on SERCA2a promoter and induced its gene and protein expression.	[54]
	Epigallocatechin-3-O-gallate (EGCG)	DNMT Inhibitor	In vitro and in vivo	HUVECs, apoE-KO mice	Jagged-1/Notch signaling pathways	Regulating Jagged-1/Notch signaling pathway	EGCG regulated Jagged-1/Notch signaling pathway and increase eNOS expression, which finally prevents ox-LDL-induced endothelial injury.	[55]
	Resveratrol	DNMT Inhibitor	In vitro	Enzymatic assays of human and bacterial DNMT, macrophage RAW264.7.	DNMT	Activating SIRT1 and inhibiting TLR4, NF-κB, HIF-1α, TGF, and ERK	Resveratrol activated SIRT1 and AMPK and inhibited TLR4, NF-κB, HIF-1α, TGF, and ERK to exert anti-inflammatory and cardioprotective effects.	[56–59]
	Folates	DNMT Inhibitor	Clinical study	120 consecutive patients with angiographically confirmed CAD and 106 healthy volunteers	C677T genes	Methylenetetrahydro-folate reductase (MTHFR)	Homocysteine plasma level was higher in patients with CAD and was correlated significantly with folic acid.	[60]
	Empagliflozin (SGLT2 inhibitor)	TET2 inhibitor	In vitro	Human ventricular cardiac myoblasts AC16 under hyperglycemic condition	TET2 (on NF-kB and SOD2 promoters)	Inhibiting TET2 binding to NF-kB and SOD2 promoters	Empagliflozin reduced TET2 binding to NF-kB and SOD2 promoters hence inhibiting demethylation on those genes and decreased the expression.	[61]
	Incretin	TET2 inhibitor	In vitro	Human aortic endothelial cells exposed to high glucose	NF-kB promoter	Inhibiting TET2 binding to NF-kB promoter	Incretin inhibited TET2 binding to NF-kB promoter which led to inhibition of demethylation and NF-kB expression.	[62]

(*Continued*)

https://doi.org/10.2147/DMSO.S463221

Table 1 (Continued).

General target	Drug name	Chemistry/ properties	Type of study/ clinical study status	Population/ subjects	Specific target	Mechanism of action	Main Results	Ref.
RNA-based gene regulation	MRG-110	Antisense Oligonucleotides	Phase I	Phase I, randomized, double-blind clinical trial included 49 participants and human arterial endothelial cells	Mir-92a-3p	Targeting MiR-92a-3p and partially inverting human arterial endothelial cells (HAECs) inflammation	MRG-110 inhibited endothelial inflammation stimulated by shear stress and oxLDL by targeting miR-92a-3p and binding to 3'UTR of KLF2 and KLF4.	[63]
	IONIS-ANGPTL3-LRx	Antisense Oligonucleotides	Phase I	44 human participants	ANGPTL3 mRNA	Impeding lipoprotein lipase by inhibiting ANGPTL3	After 6 weeks of treatment, subjects in the multiple-dose groups had lower levels of ANGPTL3 protein, triglycerides, LDL, VLDL, apolipoprotein B, and apolipoprotein C-III.	[64]
	AKCEA-APOCIIILR	Antisense Oligonucleotides	Phase I/IIa	56 healthy participants with high triglyceride levels	Hepatic APOC3 mRNA	Altering metabolism of TRL, decreasing LPL activation, and decreasing the procreation of apoC-III	AKCEA-APOCIIILR lowered the level of total cholesterol, apolipoprotein B, VLDL cholesterol, and increased the level of HDL-C	[65]
	Inclisiran	Sirna	Phase III	Patients with atherosclerotic CVD (ORION-10 trial) and patients with atherosclerotic CVD or an atherosclerotic CVD risk equivalent (ORION-11 trial) who had elevated LDL levels despite receiving statin therapy	Hepatic PCSK9 mRNA	Targeting PCSK9 which is responsible in lipoprotein metabolism	Inclisiran treatment reduced the level of LDL cholesterol by approximately 50%.	[66]
	Mipomersen	Antisense oligonucleotides	Phase III	Participants (n = 309) with LDL-C exceeding 160 mg/dL despite maximal tolerated LDL-lowering therapy	ApoB mRNA	Targeting apoB-100 mRNA	Mipomersen treatment significantly decreased the level of LDL-C up to 21%, ApoB by 22%, and Lp(a) by 27.7%.	[67,68]
	ISIS APO(a)-Rx	Antisense oligonucleotides	Phase II	64 participants with elevated Lp(a) levels	Lp(a)	Reducing Lp(a)	ISIS APO(a)-Rx treatment reduced Lp(a) levels by 66.8% in cohort A and 71.6% in cohort B.	[69]
	IONIS APO(a)-LRx	Antisense oligonucleotides	Phase I/IIa	64 participants with elevated Lp(a) levels	Lp(a)	Reducing Lp(a)	IONIS-APO(a)-LRx treatment resulted in Lp(a) reductions: 66% in the 10 mg group, 80% in the 20 mg group and 92% in the 40 mg group.	[69]
	Volanesorsen	Antisense oligonucleotides	Phase III	66 patients with familial chylomicronemia syndrome.	ApoC-III	Reducing triglyceride level by targeting apoC-III	Volanesorsen treatment led to a decrease in mean plasma apoC-III levels from baseline of 25.7 mg/dL, corresponding to an 84% decrease and lowered triglyceride levels to less than 750 mg/dL in 77% of subjects.	[70]

Abbreviations: AMPK, AMP-activated protein kinase; ApoE-KO, apolipoprotein E knockout; CVD, cardiovascular disease; DNMT, DNA methyltransferase; EGR1, early growth response protein 1; eNOS, endothelial nitric oxide synthase; ERK, extracellular-regulated protein kinase; EZH2, enhancer of zeste homolog 2; HAT, histone acetyltransferase; HDAC, histone deacetylase; HIF-1α, hypoxia inducible factor-1 alpha; hsCRP, high-sensitivity C-reactive protein; HUVECs, human umbilical vein endothelial cells; IL-1β, interleukin-1 beta; Lp(a), lipoprotein(a); LPL, lipoprotein lipase; MMP-9, matrix metalloproteinase 9; NF-κB, Nuclear factor kappa B; PCAF, cellular p300/CBP-associated factor; PCSK9, proprotein convertase subtilisin-like type 9; SGLT2, sodium-glucose co-transporters 2; SIRT1, sirtuin 1; TET, ten eleven translocation; TGF, transforming growth factor; TLR4, toll-like receptor 4; TRL, triglyceride-rich lipoproteins; sCD40L, soluble CD40 ligand; VSMCs, vascular smooth muscle cells.

rescue diabetes-induced impairment of angiogenic response through epigenetic regulation of thrombospondin-1.[71] Additionally, small-molecule BRD4 inhibitors apabetalone and JQ1, a small-molecule BET inhibitor, have been demonstrated to rescue endothelial cell dysfunction, protect monolayer integrity, and reduce Midkine expression.[72] JQ1 was the first drug developed to specifically interact with the hydrophobic pocket of the BET bromodomain, effectively blocking the interaction between multiple BET proteins (BRD 2/3/4) and acetylated histones.[73] Moreover, in vitro studies have also indicated BRD4 as an epigenetic driver of inflammation and atherogenesis, suggesting that BET inhibitors may be clinically effective in combating vascular inflammation.[35] These findings collectively underscore the potential of BET inhibitors, particularly RVX-208 and JQ1, in modulating inflammatory responses in cardiovascular diseases.

DNA Methylation-Based Atherosclerosis Pharmacotherapy

DNA methyltransferase inhibitors are divided into two categories (Table 1). Nucleoside analog inhibitors are cytosine analogs that integrate into newly synthesised DNA and sequester DNMTs through controlling their proteasomal destruction during the S phase of the cell cycle. In contrast, non-nucleoside analogs decrease the effects of DNMTs and reinduce the hypermethylated, inhibited genes by showing a high affinity for CpG-rich DNA regions.[74] Natural DNA methylation inhibitors, in addition to synthetic DNMT inhibitors, are accessible in the diet.[75]

Coa et al discovered that treating Ldlr/mice with decitabine dramatically reduced atherosclerosis by suppressing the structural movement of macrophage and adherence to ECs. Furthermore, it inhibited macrophage ER stress, thereby leading to macrophage stimulation and apoptotic initiation to reduce macrophage infiltration into atherosclerotic plaques.[51] Decitabine therapy significantly reduced atherosclerotic lesions in Apoe/mice and decreased 5-methylcytosine. Zhuang et al reported that Apoe/mice fed a Western diet had enhanced Col15A1 induction in atherosclerotic sites in vivo. This methylation reduced the expression of COL15A1, increasing the risk of illness, and was reversed by decitabine treatment which also increased COL15A1 mRNA and protein levels in SMC.[52]

RG108 inhibited DNMT3A activity, another DNA methyltransferase linked to coronary heart disease.[76] Jiang et al reported that lower shear stress accelerated the induction of DNMT3A in pig aorta and cultured human aortic endothelial cells, thereby increasing methylation of CpG islands on the promoter of kruppel-like factor 4 (KLF4) which subsequently inhibited KLF4 transcription. Interestingly, RG108 supplementation can counteract this effect by inhibiting DNMT3A thus decreasing the DNA methylation level on KLF4 promoter and inducing its expression.[53]

Hydralazine, an anti-hypertensive drug, is classified as repurposed drugs with non-classical epigenetic-based effects by which it potentially exerts cardioprotective functions.[77] Hydralazine (10 and 30 μM) may decrease DNA methylation and enhance cardiac function by raising sarcoplasmic reticulum Ca2+-ATPase (SERCA2a) and modifying calcium homeostasis in cardiomyocytes. Hydralazine decreases blood pressure by directly relaxing vascular smooth muscle.[54]

Epigallocatechin-3-O-gallate (EGCG) was demonstrated to have ability to protect vascular endothelial cells from oxidative stress, which subsequently prevents apoptosis, through induction of Nrf2/HO-1 signaling pathway.[78] A review also reported that EGCG can exert inhibition effect on LDL cholesterol synthesis, NF-κB expression and ROS production and reduce the level of plasma glucose and glycated haemoglobin and inflammatory markers, which represents anti-atherosclerosis, anti-cardiac hypertrophy, antioxidant, anti-diabetes and anti-inflammatory effects of EGCG, respectively.[79] Other study revealed that 50 μM EGCG can regulate Jagged-1/Notch signaling pathway and increase endothelial nitric oxide synthase (eNOS) expression, which finally prevent ox-LDL-induced endothelial injury.[55] Moreover, recent study demonstrated that a multifunctional liposome co-encapsulating EGCG and miR-223 decreased inflammation and lipid accumulation.[80]

Resveratrol and its derivatives can reduce DNMT3 catalytic activity. Most of its effects on gene induction are modulated by resveratrol-dependent induction of sirtuin 1 (SIRT1).[56] Resveratrol was reported to modulate lipid metabolism and inflammation hence exerting cardioprotective effects by activating SIRT1 and AMP-activated protein kinase (AMPK) and inhibiting NF-κB.[57] Recent study unraveled that resveratrol can inhibit atherosclerosis progression by downregulating toll-like receptor 4 (TLR4)/NF-κB/hypoxia inducible factor-1 alpha (HIF-1α).[58] Other researches also uncovered the effect of resveratrol on atherosclerosis through inhibiting transforming growth factor/extracellular regulated protein kinases (TGF/ERK) signaling pathway.[59]

Folate is also related to CVD epigenetics and is required for the methylation conversion of homocysteine to methionine.[60] Indeed, lower folic acid levels correspond to higher blood homocysteine levels, a metabolic risk factor for CVD. Folate insufficiency is linked to genome-wide DNA hypomethylation and is related to an increased risk of CVD. Plasma homocysteine (tHcy) is gaining attention as a CVD risk factor. In the general population, the most important genetic predictor of tHcy is the frequent C677T variation in methylenetetrahydrofolate reductase (MTHFR), which leads to increased tHcy. Plasma tHcy is especially sensitive to B-vitamins necessary for its metabolism, particularly folic acid, and to a lesser extent vitamins B12 and B6.[81]

Sodium-glucose co-transporters 2 (SGLT2) inhibitors provide a double benefit in managing CVD risk in patients with type 2 diabetes. They not only lower blood sugar levels but also appear to mitigate the development of atherosclerosis within the heart and blood vessels.[82,83] Recent study reported that empagliflozin, an SGLT2 inhibitor, can reduce TET2 binding to NF-kB and SOD2 promoters hence inhibiting demethylation on those genes which then decreases NF-kB and SOD2 expression in cardiomyocytes.[61] Furthermore, SGLT2 inhibitors could potentially improve atherosclerotic plaque stability, potentially reducing the risk of life-threatening plaque rupture.[84]

Recent research highlights the intriguing possibility that incretin-based drugs, like GLP-1 receptor agonists and DPP-4 inhibitors, may offer protection against atherosclerosis through epigenetic mechanisms. Incretin might combat vascular diabetic complications by directly modulating DNA methylation. Previous study unraveled that incretin inhibited TET2 binding to NF-kB promoter which led to inhibition of demethylation and NF-kB expression.[62] The precise mechanisms are still being elucidated; however, previous study uncovered that incretin might modulate the activity of TET2, bringing up the needs for further investigation of this drug's cardioprotective potential.[62]

Non-Coding RNA-Based Atherosclerosis Pharmacotherapy

ncRNAs significantly contribute to the development of atherosclerosis through the initiation of endothelial abnormalities, leukocyte enrollment, foam cell development, smooth muscle cell microstructure changes, and, finally, cell death promotion. Recently, some ncRNAs have been studied, for example, miRNAs, lncRNAs, etc. as a potential treatment for atherosclerosis. These drugs are classified as antisense oligonucleotides (ASO), small interfering RNA (siRNA), and miRNAs.[63–70,85] ASOs target miRNAs and block their functions, such as antagomiRs, and lock nucleic acid (LNA)-based anti-miRs, whereas siRNA acts as a mediator of the complementary mRNA degradation and miRNA imitates native double-stranded RNAs to invert the protective elements within cells.[85] Some examples of ASOs are MRG-110, IONIS-ANGPTL3-LRx, AKCEA-APOCIIILR, mipomersen, ISIS APO(a)-Rx, IONIS APO(a)-LRx, and volanesorsen, while the example of siRNA is inclisiran (Table 1).[63–70]

MRG-110 was found to target miR-92a-3p and to bind to 3′UTR of KLF2 and KLF4 which resulted in the inhibition of endothelial inflammation stimulated by shear stress and oxLDL.[63] IONIS-ANGPTL3-LRx was reported to have the ability to lower the levels of ANGPTL3 protein, triglycerides, LDL, VLDL, apolipoprotein B, and apolipoprotein C-III in human subjects after 6 weeks of treatment.[64] A phase I/IIa study investigating the efficacy and tolerability of AKCEA-APOCIIILR treatment suggested that this medication significantly reduced the level of total cholesterol, apolipoprotein B, VLDL cholesterol, and increased the level of HDL-C by targeting hepatic APOC3.[65] Phase III study of inclisiran reported that it reduced the level of LDL cholesterol by approximately 50% by targeting proprotein convertase subtilisin-like type 9 (PCSK9) which is responsible for lipoprotein metabolism.[66] Mipomersen phase III study revealed that treatment with this drug significantly decreased the level of LDL-C up to 21%, ApoB by 22%, and Lp(a) by 27.7% by targeting apoB-100 mRNA.[67,68] Phase II ISIS APO(a)-Rx study reported that this drug reduced lipoprotein(a) (Lp(a)) levels by 66.8% in cohort A and 71.6% in cohort B.[69] Indeed, treatment of IONIS-APO(a)-LRx, another drug that reduces Lp(a), also resulted in Lp(a) reductions: 66% in the 10 mg group, 80% in the 20 mg group and 92% in the 40 mg group.[69] A study about volanesorsen unraveled that this drug can decrease plasma apoC-III levels from baseline of 25.7 mg/dL, corresponding to an 84% decrease and lower triglyceride levels to less than 750 mg/dL in 77% of subjects.[70]

Figure 1 summarizes the general mechanism of action by which epigenetic drugs potentially treat atherosclerosis.

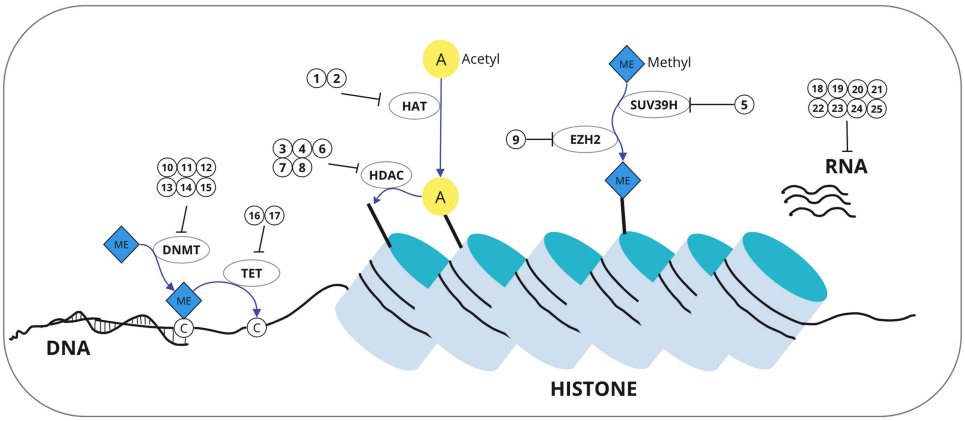

Figure 1 Epigenetic drugs in treating atherosclerosis.
Abbreviations: DNMT, DNA methyltransferase; EZH2, enhancer of zeste homolog 2; HAT, histone acetyltransferase; HDAC, histone deacetylase; 1, garcinol; 2, MG149 (anacardic acid); 3, vorinostat; 4, valproate; 5, chaetocin; 6, panobinostat; 7, romidepsin; 8, sodium butyrate; 9, GSK126; 10, decitabine; 11, RG108; 12, hydralazine; 13, EGCG; 14, resveratrol; 15, folates; 16, empagliflozin (SGLT2 inhibitor); 17, incretin; 18, MRG-110; 19, IONIS-ANGPTL3-LRx; 20, AKCEA-APOCIIILR; 21, inclisiran; 22, mipomersen; 23, ISIS APO(a)-Rx; 24, IONIS APO(a)-LRx; 25, volanesorsen.

Conclusion

Atherogenesis involves various biological pathways including those related with epigenetic changes. Numerous epigenetic drugs have been developed to specifically regulate atherogenesis-related epigenetic changes, either by modulating histone modifications, promoter CpG islands methylation, or alternating RNA-based gene regulation.

Acknowledgment

We would like to thank Universitas Padjadjaran for supporting this study.

Funding

This study was funded by Universitas Padjadjaran grant for EFA.

Disclosure

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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