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Review article

Epigenetic control of atherosclerosis via DNA methylation: A new therapeutic target?

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ABSTRACT

Atherosclerosis is a disease in which lipid-laden plaques are developed inside the vessel walls of arteries. The immune system is activated, resulting in inflammation and oxidative stress. Endothelial cells (ECs) are activated, arterial smooth muscle cells (SMCs) proliferate, macrophages are activated, and foam cells are developed, leading to dysfunctional ECs. Epigenetic regulatory mechanisms, including DNA methylation, histone modifications, and microRNAs are involved in the modulation of genes that play distinct roles in several aspects of cell biology and physiology, hence linking environmental stimuli to gene regulation. Recent research has investigated the involvement of DNA methylation in the etiopathogenesis of atherosclerosis, and several studies have documented the role of this mechanism in various aspects of the disease. Regulation of DNA methylation plays a critical role in the integrity of ECs, SMC proliferation and formation of atherosclerotic lesions. In this review, we seek to clarify the role of DNA methylation in the development of atherosclerosis through different mechanisms.

1. Introduction

Atherosclerosis is considered to be the primary cause of numerous cardiovascular diseases [1]. These disorders have been the predominant cause of mortality in the past decade [2]. During the development of atherosclerosis, slow and dynamic modifications in the cellular and molecular composition of vessel walls occur, resulting in atherosclerotic plaques [3]. Atherosclerosis is a complex disorder that is associated with the accumulation of lipids in the vessel walls, stimulation of the immune system, development of inflammatory responses (with the release of mediators, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1), oxidative stress, development of oxidized low-density lipoproteins (ox-LDLs), activation of ECs, proliferation of arterial SMCs, stimulation of macrophage and promotion of foam cells, and finally endothelial dysfunction [4–9].

Among the epigenetic regulatory mechanisms, DNA methylation is of critical importance [10]. Research investigating abnormalities of DNA methylation in atherosclerotic patients has identified a particular profile of DNA methylation and proposed various pathways and genes in the etiopathogenic mechanism of the disease (Table 1) [33]. In this article, we summarise the recent findings concerning abnormalities of DNA methylation, and their roles in the pathogenesis and progression of atherosclerosis.

2. Mechanobiology of atherosclerosis

Atherosclerosis is a complex disorder, and several contributing factors have been implicated with its development [34]. The strongest

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Table 1
Atherosclerosis-specific genes modulated via DNA methylation during the disease.

Gene	Involvement in	Ref
Insulin like growth factor 2 (IGF-II)	Cell differentiation and expansion	[11]
Paired box 6 (PAX6)	Cell differentiation and expansion	[12]
Interferon-γ (IFN-γ)	Inflammatory response	[13]
Intercellular adhesion molecule 1(ICAM-I)	Inflammatory response	[14]
Interleukin 4 (IL-4)	Inflammatory reaction	[15]
Tumor protein p53 (P53)	Apoptosis	[16]
B-cell lymphoma 2 (BCL-2)	Apoptosis	[17]
Platelet derived growth factor receptor alpha (PDGF-α)	SMCs proliferation	[18]
Estrogen receptor alpha/beta (ERα/β)	Atherosclerotic tissues remodeling	[19]
Myogenic differentiation 1 (MYOD1)	Atherosclerotic tissues remodeling	[20]
Nitric oxide synthase 3 (eNOS)	Endothelial cell remodeling	[21]
Fos proto-oncogene (c-Fos)	Shear stress	[22]
Cadherin 1 (E-cadherin)	Extracellular matrix	[23]
TIMP metallopeptidase inhibitor 3 (TIMP-3)	Extracellular matrix	[24]
Matrix metallopeptidase (MMP)-2, MMP-7, MMP-9	Extracellular matrix	[25–27]
C-C motif chemokine receptor 5 (CCR5)	Inflammation	[28]
Forkhead box P3 (Foxp3)	Inflammation	[29]
Nitric oxide synthase 2 (iNOS)	Inflammation, macrophage activation	[30]
15-Lipoxygenase (15-LO)	Plaque development	[31]
Fatty acid desaturase 2 (Fads2)	Plaque development	[22]
Superoxide dismutase 3 (SOD)	Plaque development	[32]

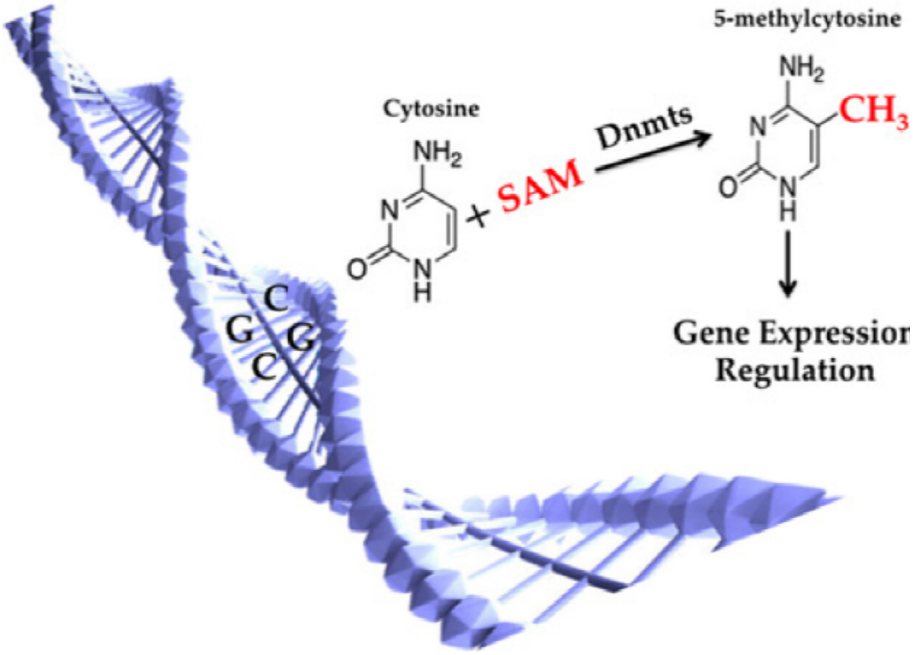

Fig. 1. Molecular mechanism of DNA methylation.
Reproduced from Zheng et al. [51].

risk factors are smoking, hyperlipidemia, male gender, diabetes, obesity, sedentary lifestyle, and aging. Hyperlipidemia has been reported to be the critical risk factor, and low lipid levels are associated with a decreased risk for the disease regardless of other risk factors [35]. It is important to note that coronary artery disease (CAD) may also develop in individuals lacking the known risk factors [36]. Genetic variations have partially explained the cumulative risk factors for CAD, and hyperlipidemia is partially explained by genetics (International Consortium for Blood Pressure Genome-Wide Association et al. [37]). Environmental contributing risk factors may be epigenetically involved in the development of atherosclerosis.

According to the 'response to injury' theory, atherosclerosis initially develops at regions with injuries to the endothelium, known as endothelial dysfunction which is characterized by decreased levels of nitric oxide (NO) in the vessel wall and enhanced generation of angiotensin II (ANGII), thromboxane, and endothelin 1 (ET1) [38]. Reduced generation of NO results in apoptosis of EC [39] and increased ANGII [40]. In arteries, lipid retention is characterized by slow thickening of the intimal layer resulting from the accumulation of modified LDL in the extracellular region of the sub-intimal layer [41,42]. Monocytes are recruited to the affected region and become macrophages, which engulf the excessive LDL and develop to foam cells, resulting in the generation of lesions [43]. Upon local injury, the SMC of vessel wall lose their function and begin to proliferate, leading to obstruction of the arterial lumen. Modified SMCs secrete mediators and thereby trigger growth of the lesion. Infiltration of immune cells leads to a local inflammatory condition. Atherosclerosis is usually regarded as an inflammatory condition, and enhanced inflammatory cytokines in the blood may be useful in the prediction of complications, such as plaque rupture, fibrosis, thrombosis, and calcification of vessel wall

[44]. These events are accompanied by elevated levels of extracellular mediators, such as vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1 (ICAM1), Monocyte chemoattractant protein 1 (MCP1), vascular endothelial growth factor (VEGF), and IL-8 [45,46]. Among the critical triggering factors of inflammatory responses are monocytes recruited to the vessel wall [47], modified LDL [48], hypomethylated self-DNA [49], and Toll-like receptors (TLRs) recognizing self RNA [50]. Inflammation seems to be present throughout the progression of atherosclerosis, and modifications to the methylation profile of DNA is thought to regulate the continuous state of inflammation.

3. Mechanisms of DNA methylation

The mechanisms underlying the regulation of gene expression through DNA methylation have previously been described (Fig. 1) [52,53]. DNA methylation is considered as an epigenetic mechanism in the mammalian cells that is mediated by DNA methyltransferase (DNMT) enzymes, and S-adenosyl-methionine (SAM) acts as the donor of methyl groups. By transfer of the methyl group (CH3) onto the C5 position of cytosines, the 5-methylcytosine (5mC) is generated [54]. Currently, five members of DNMT enzymes have been recognized that are categorized into two major groups: maintenance DNMTs (DNMT1, DNMT2) and de novo DNMTs (DNMT3a, DNMT3b, and DNMT3L). While the maintenance DNMTs are involved in the methylation of the cytosine in the hemimethylated DNA during DNA replication, the de novo DNMT enzymes play a specific role in the methylation across the embryonic development [55]. These enzymes have been attributed with reciprocal activities, in which they are involved in adding and removal of methyl groups. The methylated DNA confers a suppressed transcription state that is mediated through facilitated binding of methyl-CpG-binding domain (MBD) proteins and reduced binding of transcription factors to the methylated DNA sites. In addition, DNA methylation impresses the chromatin structure and causes the generation of co-repressor complexes. On the other hand, the unmethylated DNA confers a euchromatin structure that facilitates the binding of transcription factors to the target sites, leading to gene transcription [56].

DNA hypermethylation has been reported to occur as part of the development of numerous human disorders. In the absence of disease, DNA hypomethylation is observed in the CpG islands located in the promoter region of genes, while hypermethylation is seen in the CpG islands found within the non-promoter region of genes. Global hypomethylation of DNA, in which there is a decreased methylation level of DNA within the non-promoter regions, perhaps leads to a structural alteration and chromosome instability, resulting in transcriptional activity in undesirable sites and in normally silent (inactive) regions. That notwithstanding, global hypermethylation of DNA may be accompanied by downregulation of genes which suppress or protect against the development of diseases. As an example, decreased transcription of transposable elements, such as short interspersed nucleotide element (ALU) and long interspersed nucleotide elements-1 (LINE-1) located within the non-promoter sites, has been associated with the regulation of genome integrity by means of enhanced methylation status at their sequences [57]. In malignant diseases, a severe hypomethylation is seen in the transposable elements, conferring DNA recombination, mutations, and chromosomal instability, thereby contributing to the development of tumors [58]. DNA hypermethylation is regarded as a critical epigenetic signature seen in the promoter site of tumor suppressor genes in various malignancies [59]. Considering the shared risk factors between malignancies and atherosclerotic cardiovascular disease (CVD) [60], it has been suggested that there might be a dysregulation in the methylome and, therefore transcription, of the cardiovascular-related genes (Table 1).

4. Genome-wide DNA methylation and atherogenesis

Global DNA hypermethylation of cytosines in the CpGs has been identified in both human subjects and animal studies that was attributed to the clinical aspects of atherosclerosis [61,62]. Using genome-wide DNA methylation sequencing, a positive correlation was found between DNA methylation level and the grade of the atherosclerotic lesion in the atherosclerotic human aortas [63]. Moreover, using the methylated DNA immunoprecipitation sequencing (meDIP-seq), the differentially methylated regions were observed in the cardiovascular disease-associated genes in the ECs obtained from porcine aortas [64]. Such observations imply that the DNA methylation profiling can divulge the biomarkers of atherosclerosis, proposing a plausible role of DNA methylation in the progression of the disease.

5. DNA methylation abnormalities in atherosclerosis

5.1. Oxidative stress and DNA methylation in atherosclerosis

Oxidative stress is controlled in the body by maintaining a balance between the daily production of reactive oxygen species (ROS) and the systems which remove antioxidants. Under normal physiological conditions, a balance exists between ROS generation and enzymatic and non-enzymatic antioxidant factors, which are involved in reducing or scavenging the ROS [65]. Dysfunction of the mechanisms which remove antioxidants, or increased generation of ROS can result in a redox imbalance [66]. It has been demonstrated that prolonged oxidative stress can lead to aging and a range of disorders, including cancers, inflammation, cardiovascular disorders, and infectious diseases [67–69]. Furthermore, investigations have shown that oxidative stress during the development of atherosclerosis can modify the methylation status of DNA [70–72]. These observations followed the findings in tumor cells, that oxidative stress is associates with substantial alterations in methylation [73]. Early investigations indicated an association between 8-hydroxyguanine (8-OHdG) (a marker of ROS) and inverse alterations of DNA methylation [74]. In addition, it was reported that oxidative damage of guanines through 8-OHdG in the parental DNA strand would allow normal copying of methylation profile through pathways involved in the maintenance of DNA methylation; however oxidative damage of guanines via 8-OHdG in the newly copied DNA strand could suppress the methylation of DNA [75]. It has been reported that ROS, especially hydrogen peroxide (H_2O_2), can alter the methylation profile of DNA. H_2O_2 was shown to be able to change the DNA methylation through the facilitation of DNA methyltransferase1 (DNMT1) binding to chromatin [76]. During atherosclerosis, ROS production can alter makers of DNA methylation. Furthermore, increased methylation of the superoxide dismutase 2 (SOD2) gene, leads to its suppression and has been reported to result in SMC proliferation. Treatment with the DNMT inhibitor 5-azacytidine (5-azaC) caused upregulation of SOD2 expression and decreased SMC proliferation. The hypermethylation of SOD2, which causes decreased expression of SOD2, can stimulate the Hypoxia-inducible factor 1-alpha (HIF-1α); hence the methylation status of this gene is important for the development of atherosclerotic lesions. [19]. Little is known about the involvement of oxidative stress in the alteration of DNA methylation during atherosclerotic lesion development. That notwithstanding, due to an evident impact of oxidative stress on the modulation of DNA methylation, and considering the fact that atherosclerosis is associated with chronic oxidative stress, it has been suggested that global modifications of DNA methylation may occur during atherosclerosis.

5.2. DNA methylation during aging and atherosclerosis

Investigations have revealed that the severity of clinical presentations due to atherosclerosis is associated with aging [77,78]. In addition, it has been shown that the expression level of several genes

undergoes remarkable alterations as we age, although the mechanisms underlying these changes are not completely understood. However, it is evident that the aging process is accompanied by an alteration in the extent of DNA methylation [79]. Moreover, in vitro experiments have demonstrated that the continuous passaging of normal fibroblast leads to hypomethylation of DNA [80,81]. Additionally, aged tissues have been observed to have a similar DNA hypomethylation pattern [82–84]. Among the important genes that have been indicated to undergo a hypomethylation during aging are Estrogen receptor alpha (ERα), BMP/retinoic acid-inducible neural-specific 1 (BRINP1), E-cadherin, insulin-like growth factor-2 (IGF-2), P15, c-Fos, PAX6, c-Myc, versican, myogenic differentiation 1 (MYOD1), HIC ZBTB transcriptional repressor 1 (HIC1) [85]. Several genes that had previously been speculated to be methylated solely during tumor developments have recently been observed to be among the age-related methylation alteration genes. Clearly, aging is the primary cause of hypermethylation during malignancies [85]. Furthermore, it has been reported that the methylation of the gene coding for ERα in heart muscle occurs as a result of aging [86]. In vitro evaluation of the methylation status of SMCs demonstrated that there was a remarkable divergence in the extent of methylation of the gene coding for ERα in tissues obtained from an infant (19%) and an adult (99%) cadaver [87]. Hence, aging-associated alteration in the DNA methylation is not exclusively seen in the tumors, but might be critically involved in other age-related disorders including atherosclerosis. In spite of aging-associated global hypomethylation, gene-specific hypermethylation has also been observed that may result in an increased rate of mutations and DNA instability [88]. Thus, it seems that aging is accompanied by alteration in the DNA methylation of several genes that might also be involved in the cardiovascular system, and therefore, etiopathogenesis of atherosclerosis.

5.3. DNA methylation and inflammation in atherosclerosis

Atherosclerosis is an inflammatory condition, in which there is a systemic increased level of cytokines and recruitment of circulating leukocytes, especially monocytes to the endothelium. Monocytes reside in the sub-endothelial layer, and development into macrophages, and alter, foam cells.

A chronic inflammatory condition has been reported to stem partially from modifications in methylation status (Fig. 2). It has been shown that inflammation might be connected to a hypermethylated status of DNA and that DNA hypermethylation was related to increased mortality in patients with atherosclerosis-related disorders [89]. A global DNA methylation investigation demonstrated a strong association between an altered DNA methylation profile, and inflammation [90]. In addition, altered methylation and expression of cytochrome C oxidase subunit II (Cox-II) has been associated with inflammation in cardiovascular disease. It has been reported that Cox-II is linked with the progression of atherosclerosis, and its transcription may be triggered through pro-inflammatory mediators, including TNF-α. In addition, a negative correlation was identified between Cox-II protein and mRNA expressions and DNA methylation status. The authors suggested a link between the epigenome and the regulation of the expression of Cox-II [91]. It has been established that downregulation of cyclooxygenase-2 (COX-2) expression in subjects at high-risk and treated with aspirin may confer a protective role against the development of atherothrombosis [92]. However, when the beneficial contribution of COX-2 inhibitors is assessed, it is necessary to consider the multifaceted aspects of the prostanoid biology as well as the important role of the COX-2-derived prostaglandin I2 (PGI2) in the regulation of systemic hemodynamics that may lead to inadequate circulatory volume.

It has been reported that an inflammatory state during atherosclerosis may alter the DNA methylation of NF-κB coding gene and, therefore, result in altered signaling and the production of further inflammatory mediators that may contribute to the development of atherosclerosis [93].

Comparison of genome-wide DNA methylation of 440,292 CpG sites between human monocytes, naïve macrophages, activated macrophages with a pro-inflammatory phenotype or an anti-inflammatory state, and monocyte-derived foam cells indicated differences in methylation level between these cells. Moreover, DNA methylation highly different during monocyte-to-macrophage differentiation, that was limited to single CpGs or very short regions, and co-localized with lineage-specific enhancers [94]. These data show that localized modulation of DNA methylation at regulatory regions plays a role in cell differentiation, hence implying the involvement of DNA methylation in pathologic cell differentiation during cardiovascular disorders. Additionally, DNA methylation status of M1/M2 macrophage polarization markers was evaluated in CAD patients, resulting in the identification of differently methylated *STAT1*, *STAT6*, *MHC2*, *IL12b*, *iNOS*, *JAK1*, *JAK2* and *SOCS5* genes [95].

5.4. DNA methylation and modulation of SMCs during atherosclerosis

During the development of atherosclerosis, inflammatory mediators cause stimulation of SMCs, and their proliferation leads to plaque development. Moreover, activated SMCs generate numerous extracellular matrix components and produce a fibrous cap on the lesion [96]. The high rate of oxidized phospholipids may result in an expansion of vascular SMC (VSMC) [97–100]. Given that the VSMCs have critical roles in the development of atherosclerotic plaques, control of VSMC biology could present a promising approach to the management of atherosclerosis. Differentiation and proliferation of VSMCs occur early in the development of atherosclerotic lesions, and the developing plaques are maintained by the fibrous cap to generate a stable mass [100]. Using whole-genome shotgun bisulfite sequencing, Zaina et al. indicated that the atherosclerotic portion of the aorta was hypermethylated in numerous genomic loci. Moreover, high-density DNA methylation microarray led to the recognition of genes involved in the function of endothelial cells as wells SMCs [101]. Matrix metalloproteinases (MMPs) have been reported to be associated with VSMC biology and the progression of atherosclerosis. MMP-9 was reported to play a role in the migration of VSMCs to other organs [25–27]. In spite of MMP-9 involvement in the development of primary lesions during atherosclerosis, this enzyme can also play a role in the prevention of the development of end-stage lesions in the process of atherosclerosis [102,103]. It has been shown that VSMCs take part in the promotion of cholesterol influx, reduced rate of efflux, and development of foam cells during the early stages of development of atherosclerotic lesions. Furthermore, VSMCs undergo a process of senescence and programmed cell death that may be involved in the progression of atherosclerosis.

Reports have indicated that SMCs may develop altered expression of several genes and proteins during atherosclerosis. Profiling of the gene expression signature in the SMCs has demonstrated that several genes are underlying a transcriptional modulation of the epigenetic mechanisms, especially DNA methylation [104,105]. These genes have been observed to participate in the differentiation and phenotypic alteration of SMCs as well as the migration of these cells, leading to the development of vascular complications. A number of these genes that play a role in the differentiation of SMCs are regulated via DNA methylation. These include SMC-specific SM22α, platelet-derived growth factor (PDGF), serum response factor (SRF), ERα, and ERβ [106]. Animal studies into the proliferation of intimal SMCs indicated a global hypomethylation of DNA [32].

Moreover, investigations conducted on ApoE$^{-/-}$ mice as well as human atherosclerotic lesions demonstrated that there was a hypomethylated state of the genomic DNA [107]. A low DNMT activity, as well as global hypomethylation of DNA, was reported during proliferation and phenotypic differentiation of SMCs in vitro [108,109]. Alterations in DNA methylation affect the SMC phenotype via the extracellular matrix, which in turn results in vascular calcification [110].

In vitro experiments on human aortic smooth muscle cells and rat

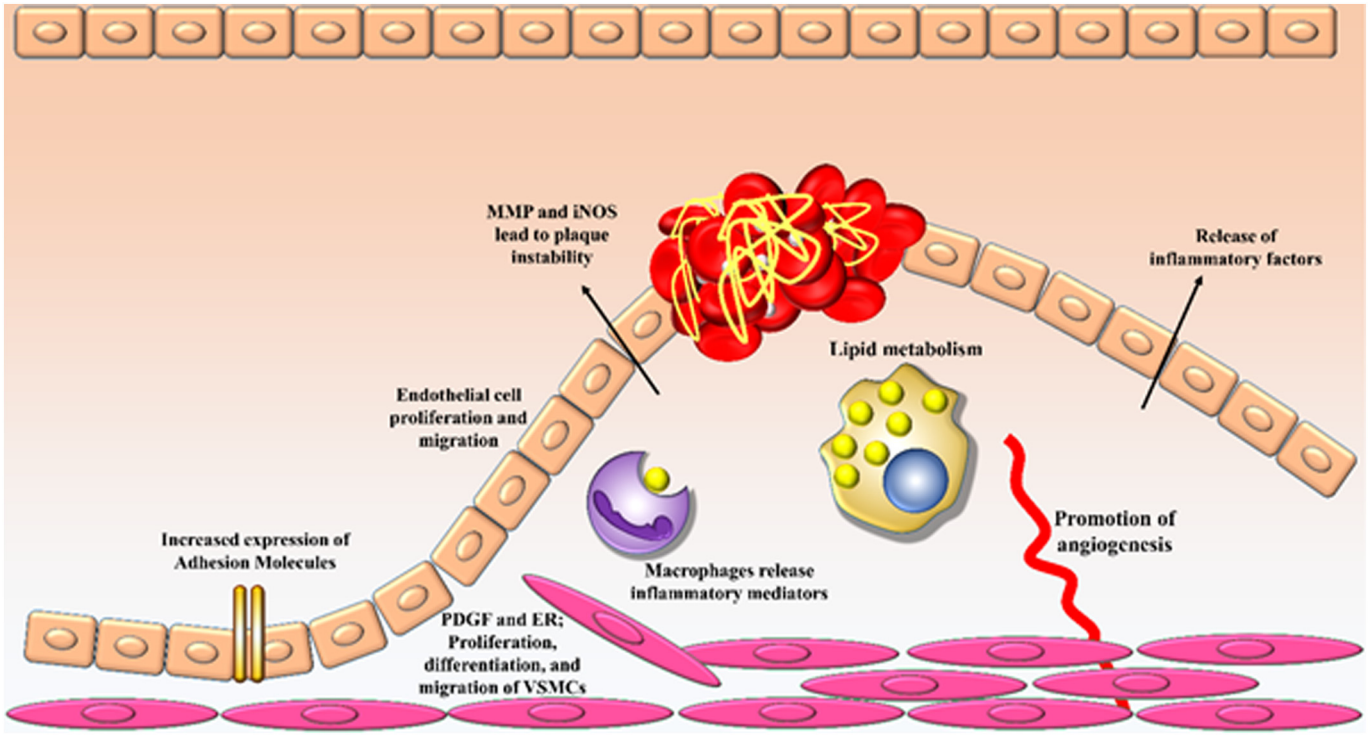

Fig. 2. DNA methylation regulation of pathogenic pathways during atherosclerosis.

aortic rings to evaluate the phenotypic difference of VSMCs triggered by high phosphate indicated that high phosphate level was associated with increased DNMT activity and methylation of the promoter region of SM22α. This resulted in a gain of the osteoblast transcription factor Cbfa1 by VSMCs. The demethylating compound procaine led to declined DNMT activity and inhibited methylation of the SM22α, which culminated in an increase in SM22α transcription and less calcification of VSMCs. Therefore, methylation of SM22α is critical in VSMC calcification [106], and might offer a pathologic pathway in atherosclerosis or CVD by impairing VSMC normal physiology.

The ten-eleven-translocation (TET) family has been implicated in modulation of the extent of DNA methylation [111]. TET proteins, containing TET1–TET3, possess DNA demethylation activity through oxidizing 5-methylcytosine (5mC) and generating 5-Hydroxymethylcytosine (5hmC). In vitro treatment of human VSMCs with 5-azacitidine (5-azaC), which is a DNA methylation inhibitor agent, resulting in the overexpression of MMP1, implying to the regulation of MMP1 in VSMCs through methylation [112]. In addition, 5-azaC was reported to inhibit the expansion and migration of SMCs in the airway by altering the methylation of PDGF as well by enhancing the contractile potential of SMCs [18]. These observations established a notion that DNA methylation of SMC might be involved in the progression of atherosclerosis. In general, DNA hypomethylation culminates in the expansion and migration of SMCs, eventuating in the expedition of plaque generation (Fig. 2).

5.5. DNA methylation and elevated homocysteinemia (eHcy) during atherosclerosis

Homocysteine (Hcy), is a non-classic sulfur-containing amino acid that is produced during the process of methionine metabolism. Hcy plays a physiologic function in DNA metabolism through methylation [113]. Methionine is converted to S-adenosylmethionine (SAM), which is the major donor of the methyl group for DNA methyltransferase enzymes. By losing methyl groups, SAM is converted to S-adenosylhomocysteine (SAH), which is a strong competitive inhibitor for methyltransferase enzymes. SAH hydrolysis leads to the generation of adenosine and Hcy [114]. Under normal physiological conditions, Hcy levels in plasma range from 5 μM to 15 μM [115]. It has been reported that elevated levels of Hcy, by modulation of the DNA methylation level, is a predisposing risk factor for the progression of atherosclerosis and plays a role in the proliferation of VSMCs as well as endothelial dysfunction. Dysregulated levels of DNA methylation, alongside with elevated levels of Hcy, has been observed in patients with CVD [116]. Reduced methylation of Alu and LINE-1 elements was reported upon incubation of VSMCs with high doses of Hcy, which increased DNMT function, increased SAH levels, and reduced SAM levels [117]. It was also observed that incubation of VSMCs with different doses of Hcy resulted in the decreased methylation and increased expression of PDGF, hence increasing the expansion of VSMC [118,119]. It has been shown that Hcy might impress the ER, which has been implicated in the pathogenesis of atherosclerosis. It was reported that there was a positive association between *estrogen receptor 1* (*ESR1*) gene prompter methylation level and the intensity of plaque lesions in atherosclerosis [120]. Studies have documented that elevated levels of Hcy may contribute to the onset of atherosclerosis development through mechanisms such as the proliferation of VSMC, stimulation of immune system, and oxidative stress [121,122]. It was also reported that incubation of monocytes with Hcy promoted the methylation of DNA in the promoter region *Peroxisome proliferator-activated receptor alpha* (*PPARA*) and *PPAR gamma* (*PPARG*) genes, culminating in a decreased levels of genes translation and transcription. Furthermore, there were a decrease and increase in the SAM and SAH levels, respectively. Such observations suggest that PPARA and PPARG gene methylations are stimulated through Hcy that might confer an important tool in the progression of atherosclerosis, proposing a potential therapeutic target for ameliorating Hcy-triggered atherosclerotic lesions [123]. Elevated level of Hcy was shown to result in DNA hypomethylation in the genes implicated in the atherosclerosis pathogenesis like *Cyclin A*, which plays a role in the inhibition of cell cycle progression as well as the endothelial remodeling. Furthermore, elevated concentrations of Hcy resulted in decreased activity of DNMT1; nonetheless, upregulation of DNMT1

reversed the inhibitory effects of Hcy on *Cyclin A* transcription and growth inhibition of EC. As a consequence, hypomethylation of *Cyclin A* through elevated levels of Hcy may be a critical mechanism that is responsible for EC proliferation ultimately leading to progression of atherosclerosis [124].

Circulating concentrations of cholesterol might also be regulated by Hcy. It was reported that clinically important levels of Hcy (100 mM) resulted in increased serum levels of cholesteryl ester, free cholesterol, and total cholesterol. Incubation of 100 mM of Hcy with human monocytes resulted in underexpression of apolipoprotein E (ApoE) [125]. Elevated levels of Hcy was shown to regulate DNA hypermethylation of ApoE and, therefore, its expression in atherosclerotic lesions [126].

Elevated levels of Hcy may contribute to the abnormal deposition of lipid in the proximal aorta, a manifestation mirroring the progress of atherosclerosis [127]. Furthermore, Hcy might alter the methylation state of genes involved in cholesterol efflux [128]. Elevated levels of Hcy may promote a global hypomethylation of DNA and, therefore, modulate the expression profile of genes implicated in atherosclerosis [114,129]. That notwithstanding, the exact molecular mechanisms underlying the DNA methylation modulation by Hcy and the association between the global DNA methylation and regulation of atherosclerosis-specific genes are not fully understood. Further investigations to resolve these issues may lead to the identification of novel therapeutic targets to treat atherosclerosis induced by Hcy.

6. Therapeutic potential of controlling DNA methylation in the treatment of atherosclerosis

Although relatively little is known concerning the DNA methylation alterations during atherosclerosis, several promising results in the field of pharmacoepigenetics have been obtained from preclinical studies and clinical trials. In a phase III trial, it was reported that apabetalone, which is an inhibitor of bromodomain and extra-terminal proteins (BET, a histone modification reader), resulted in elevated circulating concentrations of HDL, reduced CRP level, and upregulation of ApoAI, that was accompanied by fewer cardiovascular events in the patients [130]. In addition, several agents that target epigenetic regulators are now in preclinical or clinical assessments to treat cancers and may have applications in the treatment of cardiovascular events. It has been reported that Decitabine (5-aza-dC) can stimulate the expression of ESR1 and ESR2 in tumor cells and that it plays a role in maintaining the stability of anti-inflammatory phenotype in the ECs obtained from a mouse model of atherosclerosis [131]. Furthermore, decitabine was reported to be involved in the amelioration of atherosclerosis by repressing the recruitment and activation of monocytes and other immune cells [132].

7. Conclusions

Although the knowledge on the role of epigenetics in the pathogenesis of atherosclerosis is in infancy, the involvement of alterations of DNA methylation is indisputable. Additionally, other epigenetic regulatory mechanisms, including histone modifications and microRNAs, play critical roles in the etiology and pathogenesis of atherosclerosis. These epigenetic regulatory mechanisms present a promising approach to the treatment of atherosclerosis. However, to date, there is little evidence relating to the treatment of atherosclerosis by modulation of DNA methylation. Additional research is needed into the environmental stimuli of DNA methylation pattern as well as the molecular mechanisms underlying these methylation alterations implicated in atherogenesis. A more detailed understanding of the mechanisms by which abnormalities of DNA methylation result in atherosclerosis would allow the development of diagnostic biomarkers and efficient therapies. Of note, there is evidence suggesting that statins, as the major class of drugs used for the treatment of atherosclerotic cardiovascular disease, can affect epigenetic events including DNA methylation [133–135]. This finding may imply that epigenetic effects of statins can explain, at least in part, the putative anti-atherosclerotic as well as the wide range of pleiotropic activities described for these drugs [136–141]. Finally, future investigations may be directed toward epi-drugs, such as those that inhibit the DNMT enzymes, in order to treat atherosclerosis.

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Authors' contributions

All authors searched for literature and wrote and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of competing interest

Dr. Penson owns four shares in Astra Zeneca PLC and has received travel/speaker's fees from Amgen Inc. Dr. Banach has served as speakers bureau: Abbott/Mylan, Abbott Vascular, Actavis, Akcea, Amgen, Biofarm, KRKA, MSD, Sanofi-Aventis and Valeant; consultant to Abbott Vascular, Akcea, Amgen, Daichii Sankyo, Esperion, Lilly, MSD, Resverlogix, Sanofi-Aventis; received grants from Sanofi and Valeant. Other authors have no competing interests to disclose.

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