DNA Methylation, Smooth Muscle Cells, and Atherogenesis

Mikko O. Hiltunen, Seppo Ylä-Herttuala

Abstract—DNA methylation is a form of epigenetic modification of the genome that can regulate gene expression. Hypermethylation of CpG islands in the promoter areas leads to decreased gene expression, whereas promoters of actively transcribed genes remain nonmethylated. Because of cellular proliferation and monoclonality of at least some of the lesion cells, atherosclerotic lesions have been compared with benign vascular tumors. However, although genetic and epigenetic background favors neoplastic transformation, atherosclerotic plaques never develop to malignant tumors. Among cancer cells, common features are genome-wide hypomethylation, which correlates with transformation and tumor progression. Recent studies have shown that DNA methylation changes occur also during atherogenesis and may contribute to the lesion development.

Key Words: atherogenesis ■ DNA methylation ■ 5-methylcytosine ■ epigenetic gene regulation ■ gene expression

Numerous factors in plasma (eg, lipoproteins, growth factors, and monocytes) and arterial wall (eg, smooth muscle cells [SMCs], matrix, and endothelium) contribute to atherogenesis. The pathogenesis of atherosclerosis involves mainly changes in the expression and function of genes rather than mutations. Atherosclerosis begins with eccentric thickening of the intima, leading to complex lesions over several decades. Intimal thickening forms in response to almost any imaginable injury, including circulating factors and mechanical injury. Neointima is composed of SMCs, mesenchymal intimal cells, and inflammatory cells.

Before SMCs can migrate into intima, a transition in their phenotype is required. Medial nonproliferating SMCs have a contractile phenotype that enables them to regulate vascular tone. When SMCs proliferate, they acquire a synthetic phenotype. As learned from animal studies, phenotypic transition is initiated by various factors or injuries, eg, balloon denudation. The proliferative state of the SMC requires profound changes in gene expression and protein synthesis. We have shown that only a few rounds of replication of contractile medial SMCs are required to develop a significant hypomethylation of the SMC genome. Consequences of this phenomenon on gene expression are discussed later in this review. It has been shown that some intimal synthetic SMCs are of monoclonal origin, which implicates that some clones of medial SMCs have developed at least a transient growth advantage. The situation is somewhat similar to carcinogenesis, where the tumor has gained a growth advantage.

Injury to the arterial wall, such as angioplasty, induces endothelial dysfunction and stimulates SMC migration and proliferation. The highest proliferative activity of SMCs...
methylation patterns, which are then maintained by Dnmt1.12 DNA methylation comes from developmental biology, cancer biology, and studies with targeted deletions of mouse Dnmt genes.8,11,14–17 DNA hypomethylation in cancer cells occurs in highly and moderately repeated DNA sequences. These include heterochromatic DNA repeats, dispersed retrotransposons, and endogenous retroviral elements.20 In addition to cancer, we have shown recently that genomic hypomethylation is present in advanced human atherosclerotic lesions, lesions of apolipoprotein E (ApoE) knockout mice, and neointima of balloon-denuded New Zealand White (NZW) rabbit aortas (Figure 1).6 We have also shown that significant genomic hypomethylation develops during the first replications of aortic SMCs in vitro and that hypomethylation occurs in some specific genes, such as 15-lipoxygenase and extracellular superoxide dismutase.5,21 The Table shows examples of genes that may be important for atherogenesis and are at least partially regulated by DNA methylation. Because arterial SMCs are terminally differentiated, replication does not normally occur and methylation machinery is probably minimally active. It is possible that DNA hypomethylation is associated with changes in gene expression during the lesion development. This may result from a direct regulatory effect of the hypomethylation on the gene expression or be a

### DNA Methylation Changes in SMCs

DNA methylation (ie, formation of 5-methylcytosines from cytosine residues within CpG doublet by methyltransferases) has a major role as a regulator of gene expression in embryogenesis, X-chromosome inactivation in females, genomic imprinting, and carcinogenesis.8–10 DNA methylation is a form of epigenetic gene regulation that together with altered binding profile of transcription factors commonly leads to suppression of gene expression when occurring in a regulatory region.11 According to current knowledge, 3 methyltransferases are responsible for genomic methylation. Dnmt3a and Dnmt3b are responsible for de novo methylation patterns, which are then maintained by Dnmt1.12 Although elusive, there must also be some demethylase activity, because in the fertilized mouse eggs, the paternal genome is rapidly demethylated before the replication of the paternal pronucleus.13

During early development, certain chromosomal regions become methylated (de novo methylation), controlling expression of genes that regulate cell differentiation. During early stages of human carcinogenesis, genomic hypomethylation is a common phenomenon that is linked to transformation, tumor progression, and oncogene expression.14–17 In addition, it has been recently shown that hypomethylation plays a causal role in tumor formation, possibly by inducing chromosomal instability.18 The opposite situation, regional DNA hypermethylation, is present in later stages of carcinogenesis and may lead to inactivation of tumor suppressor genes.8,11,14–17 Thus, changes in genomic methylation status can lead to a selective growth advantage. Most of our knowledge about the significance of DNA methylation comes from developmental biology, cancer biology, and studies with targeted deletions of mouse Dnmt genes.19 DNA hypomethylation in cancer cells occurs in highly and moderately repeated DNA sequences. These include heterochromatic DNA repeats, dispersed retrotransposons, and endogenous retroviral elements.20 In addition to cancer, we have shown recently that genomic hypomethylation is present in advanced human atherosclerotic lesions, lesions of apolipoprotein E (ApoE) knockout mice, and neointima of balloon-denuded New Zealand White (NZW) rabbit aortas (Figure 1).6 We have also shown that significant genomic hypomethylation develops during the first replications of aortic SMCs in vitro and that hypomethylation occurs in some specific genes, such as 15-lipoxygenase and extracellular superoxide dismutase.5,21 The Table shows examples of genes that may be important for atherogenesis and are at least partially regulated by DNA methylation. Because arterial SMCs are terminally differentiated, replication does not normally occur and methylation machinery is probably minimally active. It is possible that DNA hypomethylation is associated with changes in gene expression during the lesion development. This may result from a direct regulatory effect of the hypomethylation on the gene expression or be a

### Examples of Genes Implicated in Atherogenesis That Are at Least Partially Regulated by DNA Methylation

<table>
<thead>
<tr>
<th>Gene</th>
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<tr>
<td>IFN-γ</td>
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<td>MMP-9</td>
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<td>TIMP-3</td>
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<td>ICAM-1</td>
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<td>Estrogen receptor-α</td>
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<td>EC-SOD</td>
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<td>p53</td>
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IFN indicates interferon; PDGF, platelet-derived growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; ICAM, intracellular adhesion molecule; EC-SOD, extracellular superoxide dismutase.

**Figure 1.** Hypomethylation in atherosclerotic lesions. A, 5-methylcytosine content of genomic DNA extracted from human arteries at different stages of atherosclerosis. Statistically significant (P<0.05) difference was observed when normal aortas (N; n=3) and fatty streaks (FS; n=23) were compared with advanced lesions (AL; n=29) (ANOVA and modified t test). B, 5-methylcytosine content of genomic DNA extracted from atherosclerotic lesions induced by Western diet in ApoE knockout mice. Normal control arteries were from ApoE−/− mice fed with a regular chow (P<0.05; t test). C, Genomic 5-methylcytosine contents of media and intima after denudation in normocholesterolemic NZW rabbits (media, n=2; intima, n=2) and in cholesterol-fed NZW rabbits (media, n=6; intima, n=6). Asterisks indicate statistical differences between intima and media (P<0.01; **P<0.005; t test). 5-methylcytosine analyses were done by HPLC. Reprinted with permission from Reference 6.

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- Figure 1. Hypomethylation in atherosclerotic lesions. A, 5-methylcytosine content of genomic DNA extracted from human arteries at different stages of atherosclerosis. Statistically significant (P<0.05) difference was observed when normal aortas (N; n=3) and fatty streaks (FS; n=23) were compared with advanced lesions (AL; n=29) (ANOVA and modified t test). B, 5-methylcytosine content of genomic DNA extracted from atherosclerotic lesions induced by Western diet in ApoE knockout mice. Normal control arteries were from ApoE−/− mice fed with a regular chow (P<0.05; t test). C, Genomic 5-methylcytosine contents of media and intima after denudation in normocholesterolemic NZW rabbits (media, n=2; intima, n=2) and in cholesterol-fed NZW rabbits (media, n=6; intima, n=6). Asterisks indicate statistical differences between intima and media (P<0.01; **P<0.005; t test). 5-methylcytosine analyses were done by HPLC. Reprinted with permission from Reference 6.
secondary effect by affecting DNA integrity and function. It has also been shown that regional hypermethylation occurs in atherosclerosis. Estrogen receptor-α gene was found to have an increased methylation level in atheromas compared with normal aorta.22,23 Estrogen receptor-α gene was also shown to be methylated in SMCs in vitro during the phenotypic switch.24

It has been reported that overexpression of platelet-derived growth factor, c-myc, and other growth regulatory genes occurs during atherogenesis and that SMC proliferation can be successfully inhibited by blocking the expression or activity of these genes.25–27 Several studies have shown that when culturing various cell lines in the presence of a methylation inhibitor, 5-azadeoxycytidine, expression of some inactive genes can be activated.28 Similar effects may be caused by hypomethylation in vivo. The fact that overexpression of Dnmt in cancer cannot reverse genomic hypomethylation could be explained by the lack of de novo methylation activity of the enzyme.8,29

A crucial question regarding hypomethylation is whether it is a consequence of SMC proliferation or whether it contributes to the increased proliferative activity. However, as learned from cancer cells, important consequences of DNA hypomethylation may be a decreased karyotypic stability and altered heterochromatic-euchromatic interactions favoring oncogenesis.20 It has been reported from cancer tissue that altered heterochromatic-euchromatic interactions favoring hypomethylation may be a decreased karyotypic stability and in turn to inactivation of tumor suppressor genes. In human atherogenesis, microsatellite instability occurs in 20% to 33% of cases and may be linked to the increased proliferation rate of SMCs.33,34

A special mechanism is required for the termini of the eukaryotic chromosomes (ie, telomere) to complete replication during cell division. To overcome the end-replication problem that produces gaps at the 5′ ends of the newly synthesized DNA, an enzyme called telomerase must add sequence repeats onto the preexisting 3′ overhangs of the chromosomes.35 Telomerase activity has been found in germ line cells and in cancer cells but not in somatic cells.36 This attrition of the telomere length leads eventually to the cessation of cell proliferation. Therefore, the actual biological age of the cells (ie, the number of cell divisions) may be determined by a biological clock that resides in the telomeres. Although there is abundant information about gene expression, cellular activation, and proliferation of SMCs, relatively little attention has been paid on the biological age of the vascular cells. It is hypothesized that the age of the vascular cells may have important effects on the response to injury and various growth stimuli.37 These responses may also be modulated by genomic methylation status of the vascular cells. Figure 2 presents possible contribution of epigenetic changes on SMC proliferation during atherogenesis.

Other Cancer Biomarkers and Atherosclerosis
Genomic instability (ie, chromosomal alterations and alterations in microsatellite sequences) is characteristic for human cancers, and it has been suggested that loss of mechanisms that protect genome integrity contributes to tumorigenesis.20 In normal cells, microsatellite sequence repeats are accurately maintained during replication, but in tumor cells they vary in length (ie, microsatellite instability). Although phenotypically silent, microsatellite instability indicates defects in DNA replication and maintenance machinery. These errors in the replication may lead to activation of proto-oncogenes and in turn to inactivation of tumor suppressor genes. In human atherogenesis, microsatellite instability occurs in 20% to 33% of cases and may be linked to the increased proliferation rate of SMCs.33,34

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Summary
It is concluded that hypomethylation occurs in atherosclerotic lesions and that it seems to be associated with an increased transcriptional activity. It is possible that alterations in DNA methylation may play an important role in atherogenesis and that interventions directed toward methylation machinery may be useful for the treatment of vascular disorders.

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References


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