Chapter 9

Epigenetic histone acetylation modifiers in vascular remodeling – new targets for therapy in cardiovascular disease

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ABSTRACT

Significant progress has been made in the clinical management of a variety of cardiovascular diseases. Nevertheless, the therapeutic efficacy of the current treatment modalities for atherosclerosis and restenosis is not fully sufficient in a large proportion of patients. One of the major contributing factors is the clinical and biological heterogeneity of these still life-threatening diseases, which involve processes that we do not fully understand at the moment. Over the past decades it has become increasingly clear that part of the gene-environmental interactions relevant for complex diseases is regulated by epigenetic mechanisms such as histone acetylation and DNA methylation. Epigenetic processes modulate gene expression patterns without modifying the actual DNA sequence and have profound effects on the cellular repertoire of expressed genes. They contribute to the expression of genes that play a key role in extracellular matrix formation, inflammation and proliferation, processes involved in cardiovascular pathologies such as atherosclerosis and restenosis.

Therefore, in this review we argue that epigenetic regulators involved in histone acetylating and deacetylating activities, contribute to the pathogenesis of atherosclerosis and restenosis. Furthermore, as alterations in chromatin structure are reversible, these epigenetic modifications are amendable to pharmacological intervention, which may prove to be an effective treatment modality for management of cardiovascular diseases.
1. INTRODUCTION

1.1 Atherosclerosis and restenosis
Atherosclerosis and restenosis are multifactorial processes, which have several corresponding features and share some risk factors such as diabetes and hypertension. Both processes are in a significant part characterized by an inflammatory response to injury of the endothelium, which causes reshaping of the vessel wall in size and composition, called vascular remodeling. Furthermore, they share proliferation and migration of vascular smooth muscle cells (VSMCs) and elaboration of extracellular matrix, leading to accumulation of collagen and proteoglycans.

Despite these similarities, there also are important differences in the cause and progression of atherosclerosis and restenosis. Atherosclerosis develops partly in response to elevated lipoprotein levels and cigarette smoke, whereas restenosis is mainly an overshooting wound healing response to vascular injury by balloon dilatation or stent placement. This restenotic process is not particularly sensitive to circulating lipids and smokers even seem to have a reduced risk for restenosis.1 Furthermore, atherosclerosis is, in contrast to restenosis, characterized by accumulation of oxidized lipoproteins, both within foam cells and extracellularly. While development and progression of atherosclerosis is associated with aging, the restenotic process is relatively rapidly induced after revascularization interventions such as balloon dilatation or stent placement.

Currently, atherosclerosis and restenosis are still serious health problems, which we do not yet fully understand. It is therefore important to search for factors that cause and contribute to the development of the processes involved in these diseases. Investigation into the exact mechanisms of the disturbed lipoprotein metabolism, inflammation, proliferation and migration of smooth muscle cells and vascular remodeling, will offer us more opportunities for prediction, diagnosis and finally treatment.

Because clinical risk factors do not fully predict the development of atherosclerosis and restenosis, it is important to search for genes that determine susceptibility to these risk factors. A positive family history is a major risk factor for cardiovascular disease and twin studies have shown that death from coronary artery disease at an early age of one twin is a strong predictor of the risk in the other twin.2

As reviewed by Nordlie et al., many genes in lipid metabolism, vascular homeostasis, hemostasis and inflammation have been found to be related to coronary artery disease.3 Although observed associations were not always replicated by other studies, their review emphasizes the importance of genetics in atherosclerotic disease. Moreover, the multifactorial nature of the disease suggests a role for many other, yet uninvestigated genes.

Previous research from our department has demonstrated the importance of genetics in restenosis after percutaneous coronary interventions (PCI). Polymorphisms in several inflammatory genes, such as tumour necrosis factor α (TNF-α), eotaxin, CD14,
granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin 10 (IL-10), caspase-1, but also non-inflammatory genes, such as lipoprotein lipase (LPL), stromelysin-1 (MMP3) and the β adrenergic receptor have been found to be associated with the risk for restenosis.4-7

In the past decade, research into cardiovascular diseases has been focused on the identification of these genetic factors. It has become clear, however, that part of the gene-environmental interactions relevant for complex diseases in which inflammation, proliferation and remodeling play an important role, is regulated by epigenetic mechanisms such as histone acetylation and DNA methylation.

Epigenetics, the study of non-DNA sequence-related heredity, can help to explain the relationship between an individual's genetic background, the environment, aging and disease. Thus, a review of the possible role of epigenetics in complex diseases like atherosclerosis and restenosis seems appropriate and is presented here.

1.2 Epigenetics
Epigenetic processes modulate gene expression patterns without modifying the actual DNA sequence and have profound effects on the cellular repertoire of expressed genes.8 It is well known that epigenetic processes can lead to meiotically and mitotically heritable changes in gene expression and play an important role in control of cell identity. Two well-known examples of epigenetic mechanisms are methylation of DNA at CpG dinucleotides and modification or rearrangement of nucleosomes, which include covalent post-translational modifications of histone tails.9, 10 Both processes can be influenced by environmental factors and affect gene function without changing the DNA sequence.

In contrast with classical mendelian views on inheritance, epigenetics also focuses on heredity of environmental effects which lead to DNA modifications other than DNA sequence variation, a phenomenon which is called 'epigenetic inheritance'. An increasing number of findings from both human and animal studies support the existence of epigenetic inheritance, and show that DNA-methylation is the main responsible mechanism.11, 12 Inheritance of DNA-methylation is regulated by the enzyme DNA methyltransferase 1 (Dnmt 1), which is known to copy methylation patterns during semi-conservative DNA replication.13

Although a similar mechanism has not yet been discovered for post-translational histone modifications, they are likely to be maintained through cell-division. Not only methylation of DNA, but also acetylation of histones, has been shown to accumulate in time.8 Older monozygotic twins were found to have larger differences in DNA methylation and histone acetylation when compared to younger twin pairs. This finding also demonstrates that post-translational histone modifications can be influenced by environmental factors.
Despite the lack of an evident mechanism of maintaining post-translational histone modifications during cell division, modification of histone tails is by many considered an epigenetic process.\textsuperscript{14, 15}

Epigenetic modifications of histone tails include acetylation, methylation, ubiquitination and SUMOylation of lysine residues, phosphorylation of serine residues and methylation of arginines. The various histone modifications form a code which is read by non-histone proteins and have varying effects on chromatin structure and gene accessibility.\textsuperscript{9} As a rule of thumb, conformationally relaxed chromatin (euchromatin) is a hallmark of potentially active genes and is associated with hypomethylation of CpG dinucleotides in DNA and acetylated histones. Compact chromatin (heterochromatin) is associated with transcriptionally silent genes and is associated with DNA hypermethylation at CpG dinucleotides and nonacetylated histones. These chromatin modifications are exerted by epigenetic regulators such as DNA methyltransferases (Dnmts), histone (lysine) acetyltransferases (KATs) and histone methyltransferases (HMTs), which are increasingly being implicated as direct or indirect components in the regulation of expression of vascular, immune, and other (tissue)-specific genes.

A great promise of epigenetics is that it offers new targets for therapy in cardiovascular disease. Epigenetic processes are reversible by nature, which is underscored by the counterbalancing action of KATs and KDACs (lysine deacetylases), and of HMTs and HDMs (histone demethylases), and is required for fine-tuning of gene expression for fundamental cellular processes such as cell proliferation and differentiation. This offers the prospect of pharmacological inhibition of the various enzymatic activities involved in epigenetic DNA and histone modifications, which is aimed at the induction or silencing of transcription of disease relevant genes. In addition to the possibility of modifying the effects of deleterious genes, it might be possible to influence the effects of environmental risk factors.

Since KATs and KDACs are involved in fundamental processes that regulate the expression of multiple genes, they most likely play an important role in the multifactorial processes that lead to atherosclerosis and restenosis. Therefore, it is important to identify those KATs and KDACs that play a role in the transcriptional regulation of genes, the products of which contribute to the processes involved in neointima formation and atherogenesis, such as inflammation, smooth muscle cell proliferation and matrix formation.

Single gene disorders in the histone acetylation machinery have already been shown to cause clinical syndromes. For example, patients with Rubinstein-Taybi syndrome are known to have mutations in the gene encoding CREB binding protein (CBP), a transcriptional coactivator with KAT-activity.\textsuperscript{16} Therefore it seems likely that also polymorphisms in genes encoding
KATs or KDACs, provided that they have functional implications, may influence cardiovascular disease susceptibility by affecting the fidelity of these enzymes. This hypothesis was recently confirmed by research from our department showing that the -2481 G/C polymorphism in the promoter of the gene encoding p300/CBP-associated factor (PCAF), a KAT with an important role in inflammatory gene activation, is significantly associated with all-cause mortality in the PROSPER-study (n=5804) and with clinical restenosis after a percutaneous coronary intervention in the GENDER-study (n=3104). Genetic epidemiology may lead to the identification of more risk markers in genes encoding KATs or KDACs and provide new insights into the pathophysiology of atherosclerosis and restenosis, which could contribute to the development of better therapy.

In this review we will discuss the role of KATs and KDACs in the transcriptional regulation of genes that play a critical role in inflammation, proliferation and matrix formation, processes which are thought to be pathognomonic for atherosclerosis and restenosis development.

2. KATS AND KDACS

Gene expression is regulated by acetylation of core histones through the action of KATs by transfer of an acetyl group to the ε-portion of lysine residues. Hyperacetylation of histones results in an open modification of chromatin structure and affects gene transcription through accessibility of DNA to the basal transcription initiation machinery (figure 1).\textsuperscript{17-19} Conversely, gene repression is mediated via KDACs and other corepressors, which remove the acetyl groups from hyperacetylated histones and counteract the activity of KATs resulting in a closed chromatin structure. These nuclear enzymes have been shown to regulate the expression of inflammatory genes by modifying chromatin structure.\textsuperscript{20} KATs are recruited to promoters by gene regulatory proteins that interact with specific recognition sequences in DNA, and in this way control gene transcription. This is exemplified by the transcriptional control of MHC-II genes in which the class II transactivator (CIITA), a component of the promoter assembled enhanceosome, provides the platform for recruitment of KAT and KDAC activities.\textsuperscript{21-23}

At the moment a large variety of KATs have been identified, which can be divided in several families, including the PCAF/Gcn5, p300/CBP, MYST, SRC, TAF\textsubscript{II}250, KAT1 and ATF-2 families. The KATs within these families show high sequence similarity, but there is poor similarity between these families.\textsuperscript{24} All KAT proteins vary in their KAT-domains and their substrate-specificity, but have the common feature that they require the assembly of multiprotein complexes for nucleosomal acetylation.\textsuperscript{25} Their precise substrate-specificity even depends on the context of these multisubunit KAT-complexes.\textsuperscript{26}
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The KDAC enzymes are zinc-dependent aminohydrolases which can be grouped into different classes. 27 Class I KDACs (KDAC 1-3 and 8) are widely expressed, reside almost exclusively in the nucleus and are known to modulate cell proliferation and survival. Class II KDACs (KDAC 4-7, 9 and 10) have a more restricted distribution, are able to move between the nucleus and cytoplasm in response to certain cellular signals and may be involved in cellular differentiation.27, 28 Class II KDACs can be further divided in subclasses IIa (KDAC 4, 5, 7 and 9) and IIb (KDAC 6 and 10). Class IIa members form a distinct group because of the presence of an extended N-terminal regulatory domain, 29 whereas KDAC 6 and 10 contain an extra catalytic domain. 30 The most recently discovered isoform is KDAC11, which constitutes class IV.31 A different NAD+-dependent family consists of sirtuins (SIRT 1-7) which are thought to be involved in apoptosis of mononuclear cells.32, 33

The different KDACs are likely to be regulated differently. KDACs interact with corepressor molecules, such as nuclear receptor corepressor and ligand-dependent corepressor, NuRD and mSin3, all of which aid KDACs in gene repression and may provide specificity by selecting which genes are switched off by KDACs.34, 35 Furthermore, KDAC activity is associated with the action of Polycomb Group Proteins,36 which play a key role in gene silencing and maintenance of cellular identity.

Besides their role in histone acetylation, KATs are also found to act as factor acetyltransferases (FATs), which acetylate many non-histone proteins,37 such as p53, which

Figure 1. Histone acetylation
Acetylation of histone-tails is mediated by histone (lysine) acetyltransferases (KATs) and results in an open modification of chromatin structure. It allows transcription factors to access the DNA and initiate gene transcription. Conversely, gene repression is mediated via lysine deacetylases (KDACs), which remove the acetyl groups from the histone-tails, resulting in a closed chromatin structure.
is directly acetylated by PCAF, CREB binding protein (CBP) and p300, and nuclear factor kappa B (NFκB), which is acetylated by CBP and p300. Acetylation of these proteins often results in increased DNA-binding and transcriptional activity. Accumulating evidence suggests that many KDACs can deacetylate these non-histone proteins. For example, KDAC3 is known to deacetylate the p65 subunit of NFκB, promoting association with IκBα, which leads to IκBα-dependent nuclear export of NFκB. Furthermore, KDAC1 has been shown to deacetylate p53 in vitro and in vivo. As many other non-histone proteins are also known to be targeted by KATs and KDACs, their influence on gene expression is not necessarily dependent on chromatin remodeling. Changes in gene expression which are caused by alterations in the activity of KATs or KDACs can therefore not automatically be regarded as epigenetic.

Over the past years several KAT and KDAC inhibitors have been identified. These inhibitors mechanistically affect the action of KATs and KDACs. The large variety of KDAC-inhibitors that have been discovered or developed can be divided in structural classes (table 1). Early phase clinical trials have demonstrated that inhibitors from all different classes seem to be well tolerated and exhibit clinical activity against several human neoplasms.

In the next paragraphs we will discuss the role of KATs and KDACs in inflammatory, proliferative and remodeling processes associated with atherosclerosis and restenosis. Furthermore, we will discuss the potential applicability of KAT and KDAC inhibitors in disease management.

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<th><strong>Table 1. Structural classes of KDAC inhibitors</strong></th>
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<td><strong>Short-chain fatty acids</strong></td>
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<td><strong>Hydroxamic acids</strong></td>
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<td><strong>Benzamides</strong></td>
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<td><strong>Epoxyketone-containing cyclic tetrapeptides</strong></td>
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Adapted from: Santini et al. (2007), Drummond et al. (2005) and Monneret et al. (2005).
3. INFLAMMATION, THE ROLE OF KATS AND KDACS

3.1 Nuclear Factor kappa B
Atherosclerosis and restenosis are for a major part determined by inflammation.\textsuperscript{5, 48} The expression of multiple inflammatory genes is regulated by pro-inflammatory transcription factors, such as NFκB. The transcription factor NFκB plays a role in the orchestration, amplification and perpetuation of the inflammatory response and forms the molecular basis of chronic inflammation.\textsuperscript{49-51} KATs and KDACs have been implicated in modulating NFκB activity. In unstimulated cells NFκB is found in the cytoplasm associated with its inhibitor protein called IκB, which masks the nuclear translocation signal and prevents NFκB from entering the nucleus.\textsuperscript{52} Upon cell stimulation with various NFκB inducers, including reactive oxygen species (ROS) and lipid peroxidation products, IκB is rapidly phosphorylated and thereby targeted for ubiquination.\textsuperscript{53, 54} The released NFκB dimer is translocated to the nucleus and activates target genes by binding with high affinity to κB elements in their promoters.\textsuperscript{55, 56} This process is known to be activated by KATs and repressed by KDACs.\textsuperscript{57} IL-1β and TNF-α have been shown to stimulate the binding of the NFκB p65 subunit to CBP and to induce histone acetylation, thus leading to an increase in NFκB-mediated expression of inflammatory genes, such as GM-CSF and IL-8 (figure 2).\textsuperscript{58, 59} Furthermore, p300 has been shown to enhance angiotensin II-induced IL-6 expression in rat VSMCs, a process which is also mediated by NFκB.\textsuperscript{60} Also PCAF is known to be required to coactivate p65-dependent transcription and has been shown to activate the transcription of several NFκB-regulated genes known to be involved in cardiovascular disease. Spermidine/Spermine N1-Acetyltransferase 2 (SSAT2) has been found to cooperate with CBP and PCAF to enhance TNF-α-induced NFκB activity.\textsuperscript{61} Furthermore, Miao \textit{et al.} demonstrated that PCAF could enhance the p65 mediated increase in TNF-α promoter activity and that high glucose increased the recruitment of PCAF to the TNF-α and cyclooxygenase 2 (COX-2) promoters.\textsuperscript{62} They demonstrated concomitant acetylation of specific lysine residues of histone H3 and H4 at these promoters. Since TNF-α and COX-2 have been implicated in the development of both atherosclerosis\textsuperscript{63, 64} and restenosis,\textsuperscript{4, 65} PCAF may play a role in the development of these diseases. A role for PCAF in vascular disease was recently confirmed by findings from our department showing that the -2481 G/C polymorphism in the promoter of the PCAF gene is significantly associated with all-cause mortality in the PROSPER-study (manuscript in preparation, abstract published in Circulation, Oct 2007; 116: II_40). The minor C allele was associated with a significant survival advantage, while this allele was also found to protect against clinical restenosis after a percutaneous coronary intervention in the GENDER-study.

In line with the findings showing a role for KATs in NFκB-mediated transcriptional activation, KDACs have been shown to reverse this process and to repress NFκB-mediated
Furthermore, KDAC-inhibition by trichostatin A (TSA) was found to increase basal and TNF-α-induced expression of the NFκB-regulated IL-8 gene, whereas expression of KDAC1 and KDAC2 repressed TNF-induced NFκB-dependent gene expression.

In addition to its ability to associate with KATs and KDACs, the p65 component of NFκB is also a direct target for acetylation and deacetylation. PCAF and CBP/p300 appear to acetylate specific lysine residues on the p65 subunit of NFκB, increasing its DNA-binding and causing transcriptional activation also by this mechanism. PCAF and CBP/p300 appear to acetylate specific lysine residues on the p65 subunit of NFκB, increasing its DNA-binding and causing transcriptional activation also by this mechanism. PCAF and CBP/p300 appear to acetylate specific lysine residues on the p65 subunit of NFκB, increasing its DNA-binding and causing transcriptional activation also by this mechanism.

**3.2 Granulocyte Macrophage – Colony Stimulating Factor**

Several findings indicate the importance of GM-CSF in atherosclerosis and restenosis. Administration of GM-CSF has been shown to prevent the progression of athero-
sclerosis in WHHL (Watanabe heritable hyperlipidemic) rabbits. Furthermore, in a rabbit model of restenosis, GM-CSF injections have been shown to reduce neointima formation. In further support of a role for GM-CSF in the development of restenosis, variation in the GM-CSF gene has been shown to be related to the risk of restenosis in a large patient population undergoing PCI. Because of its role in atherosclerosis and restenosis it is of interest to note that the NFκB-mediated, inflammatory signalling induced hyperacetylation of histone H4 also correlated with an increased expression of GM-CSF. The KDAC inhibitor TSA was found to lead to an increased expression of GM-CSF and IL-8 in alveolar macrophages and airway epithelial cell lines after activation with inflammatory stimuli.

3.3 Eotaxin
Eotaxin, an eosinophil chemoattractant important in inflammatory responses, has already been shown to play a role in atherosclerotic disease. Eotaxin plasma levels were found to be associated with angiographic coronary artery disease and human atherosclerotic plaques were found to express increased quantities of eotaxin mRNA. Furthermore, the A23T polymorphism in the eotaxin gene is associated with the risk for myocardial infarction and the -1328 A allele was found to decrease the risk for restenosis after PCI.

Inflammatory signalling by TNF-α induces selective histone H4 acetylation and binding of p65 to the eotaxin promoter as determined by chromatin immunoprecipitation (ChIP), resulting in eotaxin gene transcription. Notably ChIP analysis also revealed that Beta(2)-agonists and glucocorticoids were able to reduce transcription of eotaxin by inhibiting both TNF-α-induced histone H4 acetylation and recruitment of p65 to the eotaxin promoter, without altering the capability of NFκB to translocate to the nucleus.

3.4 Cyclooxygenase-2
Several proteins that have been implicated in the development of cardiovascular disease have been studied more extensively in cancer and inflammatory diseases. This is exemplified by Cox-2, a protein that may be associated with the development of atherosclerosis and restenosis, which plays an important role in colon carcinogenesis and inflammation. Cox-2 expression can be induced by several cytokines, including TNF-α, and the KDAC-inhibitors sodium butyrate (NaBu) and TSA were found to repress Cox-2 mRNA and resulting protein synthesis in a colon cancer cell line (HT-29). Although KDAC inhibition by NaBu is known to suppress cytokine-induced NFκB activation, and NFκB binding sites exist within the Cox-2 promoter, this repression of Cox-2 expression in HT-29 cells was not mediated by a lower Cox-2 promoter activity, but was related to mRNA stabilization. These observations infer that besides a direct effect of KDAC
inhibitors on gene promoter activity, additional cellular mechanisms exists which contribute to modulation of mRNA expression and protein levels of Cox-2.

3.5 Reactive Oxygen Species
Activated inflammatory cells and a perturbation of lipoprotein metabolism are key factors in atherogenesis. The oxidative modification of lipids is involved in the recruitment of mononuclear leukocytes to the arterial intima. Furthermore, activated leukocytes, as well as endothelial mitochondria, can produce reactive oxygen species (ROS) that are associated with endothelial dysfunction. Elevated intracellular ROS are generated under various physiological and pathological conditions involving oxidative stress, including inflammation, ischemia, reperfusion, and sepsis. The biological effects resulting from oxidative stress are in part mediated by modifications in KAT and KDAC activities, which lead to alterations in histone acetylation levels and resulting NFκB activation. Several in vitro experiments have shown that hydrogen peroxide increases KAT activity and decreases KDAC activity, leading to an increase in histone acetylation and expression of inflammatory mediators.

The role of ROS in chromatin remodeling is further illustrated by the observed imbalance in expression levels of KATs and KDACs in bronchial biopsies from patients with asthma, when compared with airways of non-asthmatic subjects. Notably, treatment with inhaled steroids resulted in increased KDAC activity and reduced KAT activity in subjects with asthma thereby influencing this imbalance. This is in accordance with other studies describing that recruitment of KDAC activity to activated transcriptional complexes was required for glucocorticoid repression of inflammatory genes. In addition, the glucocorticoid receptor also acts as a direct inhibitor of CBP-associated KAT activity. This could be a mechanism that explains the effectiveness of glucocorticoids in suppressing inflammation in asthma.

Together there are several lines of evidence that suggest an important role of altered histone acetylation levels in inflammatory processes, which are associated with atherosclerosis and restenosis.

4. PROLIFERATION OF (V)SMCS AND HISTONE ACETYLATION

4.1 Serum Response Factor
Precise control of smooth muscle cell (SMC) gene transcription plays a major role in vascular development and pathophysiology. Vascular SMCs undergo profound changes in their phenotype during neointima formation in response to vessel injury or within atherosclerotic plaques. Serum Response Factor (SRF) is an important mediator in transcriptional activation of genes that exhibit SMC-restricted expression. SRF exerts
its activation through binding to CARG box DNA sequences found within regulatory elements of these genes. Recent findings indicate that binding of this factor is influenced by chromatin structure. In vitro and in vivo experiments have shown that SRF-binding is associated with patterns of posttranslational histone modifications that are specific to the SMC lineage, including di-methylation of lysine residues 4 and 79 in histone H3, acetylation of lysine 9 in histone H3 and acetylation of histone H4. In particular, deacetylation of histone H4 was coupled with loss of SRF binding to CARG box DNA during suppression of SMC differentiation in response to vascular injury. SRF-mediated transcriptional activation is enhanced by myocardin, which acts as a co-activator, through interaction with the MADS box of SRF. Myocardin is specifically expressed in cardiac and smooth muscle cells, and plays a critical role in the differentiation of SMCs through activation of SMC-specific genes by tethering to CARG boxes with SRF. The promyogenic activity of myocardin is enhanced by p300, a lysine acetyltransferase that associates with the transcription activation domain of myocardin. Conversely, class II lysine deacetylases interact with a domain of myocardin distinct from the p300-binding domain and suppress smooth muscle gene activation by myocardin.

4.2 Hyper nuclear acetylation

Hyper nuclear acetylation (HNA) plays a role in proliferation, differentiation and apoptosis. Using an antibody against ε-acetylated lysine, increased histone acetylation was observed in VSMCs in coronary atherosclerotic lesions, whereas it was not present in normal coronary arteries. Furthermore, thrombin, a humoral factor known to cause activation and proliferation of VSMCs, strongly potentiates HNA in cultured VSMCs. In thrombin-induced HNA of VSMCs, the MAP kinase pathway and the CREB binding protein (CBP) are implicated. It suggests that coactivators cooperating with signal-dependent transcription activators play a role in atherosclerogenesis via HNA in VSMCs.

4.3 Cell-cycle regulators

Because KDACs also modulate histone acetylation levels at the site of genes involved in cell cycle control, they have also been implicated in the proliferation of SMCs. This is illustrated by the observation that TSA was found to inhibit SMC proliferation via induction of p21waf1. Activity of p21waf1 is known to induce cell-cycle arrest in vascular smooth muscle cells, and A20, a NFκB-dependent gene that has been shown to inhibit proliferation of VSMCs via increased expression of p21waf1, has been shown to prevent neointima formation after balloon angioplasty in a rat model of carotid artery stenosis. Also PCAF could play a role in the suppression of VSMC proliferation by its ability to directly acetylate p53. PCAF has been shown to activate p53-responsive enhancer
elements within the p21waf1 promoter.\textsuperscript{39} Moreover, KDAC8 was found to be exclusively expressed in cells showing smooth muscle differentiation, including visceral and vascular smooth muscle cells, myoepithelial cells and myofibroblasts, and is mainly detected in their cytosol.\textsuperscript{98} Until recently, it was thought that its activity was restricted to the nucleus, with histones as a unique substrate.

5. REMODELING: EXTRACELLULAR MATRIX FORMATION AND CHROMATIN DYNAMICS

Inappropriate remodeling of the vessel wall in response to a variety of stimuli, including hemodynamic changes, inflammation and tissue injury, is currently thought to be causative to the development of atherosclerosis and restenosis. The matrix metalloproteinases (MMPs) comprise a family of structurally related proteins, which includes collagenases, stromelysin and gelatinases. They have the collective capability to degrade components of the extracellular matrix and to act as mediators in a variety of cell signalling pathways through degradation of non-matrix proteins. MMP expression is strongly enhanced in vascular pathologies and is therefore thought to contribute to the disease process. This is illustrated by the observation that enhanced expression of MMP2 and MMP9 (also referred to as Gelatinase A and Gelatinase B) is noted in vulnerable regions of human carotid plaques in association with macrophages.\textsuperscript{99} Furthermore, as reviewed by Newby, induction of MMP9 and activation of MMP2 occur rapidly after balloon injury in multiple animal models.\textsuperscript{100} Additional evidence for an important role of MMP2 and MMP9 in vascular remodeling comes from knockout mouse-models of these gelatinases which showed reduced VSMC migration and less neointima formation after arterial injury.\textsuperscript{101-104} Increased MMP3 (also referred to as stromelysin-1) expression has been linked to plaque rupture.\textsuperscript{99,105} In addition, several studies investigating the MMP3 5A/6A promoter polymorphism have shown that the risk of myocardial infarction is higher in patients carrying the 5A allele, which is associated with increased MMP3 levels.\textsuperscript{106-110} MMP1 and MMP13 are also associated with cardiovascular disease. MMP1 was found expressed in human atherosclerotic lesions and not in unaffected blood vessel walls.\textsuperscript{111} Moreover, the MMP1 1G/2G polymorphism at position -1607 of the transcriptional start site, which is associated with a higher promoter activity, reduces the risk for coronary artery disease.\textsuperscript{111} Likewise, the MMP13 -77 A/G polymorphism is associated with atherosclerosis in the abdominal aorta of black males.\textsuperscript{112}

Due to their essential role in vessel wall remodeling, MMPs could become prime targets for pharmacological interference in various pathological conditions that exhibit increased or decreased MMP levels and resulting excessive or reduced matrix degradation.
In the next sections we will discuss the role of the chromatin environment in expression of the various MMPs in vascular pathologies.

5.1 MMP2 and MMP9 (Gelatinase A and Gelatinase B)
Epigenetic mechanisms have been implicated in regulation of transcription and/or functional expression of MMP2 and MMP9. This is underscored by several observations showing modulation in expression levels of MMPs following exposure of cells to different KDAC inhibitors in various cell types. Treatment of different tumour cell-lines with NaBu resulted in modulation of secreted expression levels of MMP2 and MMP9, which was accompanied by global histone H4 hyperacetylation. On the other hand it was demonstrated that increased histone acetylation by TSA-treated 3T3 cells decreases mRNA as well as zymographic activity of MMP2. This TSA-induced inhibition of MMP2 activation is mediated via upregulation of the glycoprotein RECK. With respect to MMP9, it has been demonstrated that IFN-γ induced a reduction in MMP9 expression, which is mediated by CIITA. CIITA is induced by IFN-γ through the JAK/Stat signalling pathway and downregulation of MMP9 is exerted by CIITA through sequestering of CBP from the MMP9 promoter, effectively reducing histone H3 acetylation, explaining the inhibition in MMP9 transcription by IFN-γ. Further support for a role of epigenetic mechanisms in the regulation of MMP9 expression comes from the observation that the metastases associated gene MTA1 represses MMP9 expression, in part by the recruitment of KDAC2 to the distal MMP9 promoter-region and modulation of the activity of MMP9 by depsipeptide in uveal melanoma cells.

5.2 MMP3 (Stromelysin-1)
Besides MMP2 and MMP9, several findings also indicate the involvement of epigenetic mechanisms in the expression of MMP3. Overexpression of the TEL (Translocation-ETS-Leukemia)-protein, which specifically associates with KDAC3, represses transcription of the stromelysin-1 gene. A role for KDAC3 in the TEL-mediated repression was supported by the observations that histone H3 was underacetylated near the TEL binding sites in the stromelysin-1 promoter and that administration of TSA impaired TEL-dependent repression of the stromelysin-1 promoter. A role for histone acetylation in MMP3 expression is further supported by the finding that NaBu selectively enhanced protein production and mRNA expression of stromelysin-1 in TNF-α or IL-1β stimulated mesenchymal cells. Furthermore, it has been demonstrated that the transcription factors Ets-1 and Ets-2 recruit p300 and CBP to the human stromelysin-1 promoter to activate transcription.
5.3 MMP1 and MMP13 (Collagenase 1 and collagenase 3)
Histone acetylation modifications are also implicated in the expression of MMP1 and MMP13. This notion is derived from studies in chondrocytes which revealed that exposure of cells to TSA or NaBu blocks the induction of MMP1 and MMP13 by pro-inflammatory cytokines at both the mRNA and protein level.\textsuperscript{122} Furthermore, NaBu treatment of liver cancer cells results in decreased transcription of MMP1.\textsuperscript{123} Additional evidence for a role of histone acetylation modifications in the control of MMP1 expression is derived from the observation that the co-activator p300 has been shown to relieve the Smad-mediated inhibitory effect of TGF-β in cytokine-induced MMP1 expression in dermal fibroblasts.\textsuperscript{124} Expression of MMP1 is also indirectly affected by TSA and SAHA (Suberoylanilide hydroxamic acid) through inhibition of c-jun.\textsuperscript{125}

Together, histone acetylation modifications have been shown to play a direct and indirect role in the regulation of MMP expression, critical mediators in vascular remodeling and as such implicated in the pathology of atherosclerosis and restenosis.

6. CONCLUSIONS AND FUTURE DIRECTIONS

Besides genetic control, epigenetic mechanisms contribute to expression at the transcriptional level of genes, the products of which play a key role in extracellular matrix formation, inflammation and proliferation as discussed in this review. Altered expression levels of these genes have been observed in cardiovascular pathologies and therefore it is thought that these aberrant expression patterns contribute to the processes associated with atherosclerosis and restenosis. As the nucleotide composition of genes is not altered by these epigenetic processes and alterations in chromatin structure are reversible, these epigenetic modifications are amendable to pharmacological intervention, which may prove to be an effective treatment modality for the management of cardiovascular diseases.

Although chromatin modifications exist at the level of DNA methylation at CpG dinucleotides and a variety of post-translational histone modifications, we have focussed on the role of histone acetylation modifications exerted by KATs and KDACs in cardiovascular disease. Besides accumulation of epigenetic modifications in aging, histone acetylation modifications are also dynamic: rapid alterations in the levels of histone acetylation modifications can be measured after cell activation by a variety of external stimuli. It can therefore be envisioned that similar alterations in histone acetylation modifications are induced in the response to cellular injury such as inflicted by e.g. flow shear stress or by balloon angioplasty. As detailed in the various preceding sections of this review, there is ample experimental evidence from different cellular models that indeed the activities of KATs and KDACs are key elements, either directly or indirectly,
in the transcriptional control of vascular disease-associated genes. Aberrant expression of these chromatin modifying activities therefore has a profound effect on the overall level of transcription of disease-associated genes and function of their products in the processes involved in extracellular matrix formation, inflammation and proliferation.

Clinical feasibility of applying drugs which inhibit the activity of KATs and KDACs is demonstrated in the application of these compounds in the treatment of cancer and neurological disorders. However, it should be realized that application of these compounds can also result in non-specific activation of genes and other genomic elements not only in diseased cells but also in normal cells. Especially the use of non-specific inhibitors that target many different KATs or KDACs can be expected to have broad effects on gene expression and may inflict potential harmful side-effects. It is therefore important to identify those KATs and KDACs that play a role in the transcriptional regulation of cardiovascular disease-associated genes and to develop specific inhibitors that target these KATs and KDACs. As also a single KAT or KDAC family-member can be involved in the transcription of multiple genes, even specific inhibitors could have unwanted effects on gene expression. However, each of these family-members could have some degree of specificity to a set of genes acting together in a vascular disease-associated process. Although KATs and KDACs are known to be substrate-specific, it is thus far not known if they can also be specific to certain processes.

Nevertheless, many KDAC-inhibitors appear to be well tolerated in patients and local application of these epigenetic modifiers would overcome the side-effects which are inherently linked with systemic application.

Several recent investigations indeed indicate that KDAC-inhibition may be beneficial in vascular disease-associated processes. Transcription of the \textit{CYP7A1} gene encoding cholesterol 7α-hydroxylase, an enzyme involved in the intra-hepatic conversion of cholesterol to bile acids, is known to be repressed by bile acids as part of a feedback regulatory mechanism. Recent findings demonstrate that the bile acid-mediated repression of this gene was caused by an increase in the nuclear concentration of KDAC7, which promoted the assembly of a repressive complex at the \textit{CYP7A1} promoter. Notably, treatment of LDL-receptor knockout mice with the KDAC-inhibitors TSA and valproic acid elevated \textit{Cyp7a1} mRNA levels and consequently reduced total plasma and LDL-cholesterol dramatically. In addition to these findings, \textit{in vitro} experiments showed that TSA strongly inhibited SMC proliferation and TNF-α production by activated macrophages, processes with an important role in atherogenesis. Interesting in this context is the recent finding that also statins inhibit KDAC-activity. Lovastatin was found to induce p21 expression through dissociation of KDAC1/2 and association of CBP, leading to histone-H3 acetylation at the site of the p21 promoter. Therefore, part of the beneficial effect of statins in vascular disease might be due to its role in these mechanisms.
Recent findings from Granger et al. also identify KDACs as possible targets for therapy in cardiovascular disease. They showed, utilizing a standard murine model of ischemia-reperfusion, that ischemia induces KDAC activity in the heart resulting in deacetylation of histones H3 and H4.129 Within a treatment window from 1 hour before ischemia until 45 minutes after reperfusion, the KDAC inhibitors TSA and Scriptaid were able to reverse the induction of ischemia-induced KDAC activity in vivo and to reduce myocardial infarct size by approximately 50%. Furthermore, a 5 hour period of hypoxia induced a strong decrease in acetylated histones H3 and H4 in mouse cardiomyocytes in vitro, which could be completely blocked by TSA. The finding that KDAC-inhibition can reduce the size of myocardial infarction in mice by half, even when treatment is applied after reperfusion, has important implications for the potential application of these compounds in the context of acute coronary syndromes.

In conclusion, over the past years it has become increasingly clear that besides genetic also epigenetic components contribute to the etiology of cardiovascular disease. Dynamic interactions of the environment and the epigenome determine accessibility of genes which have profound effects on their transcription and function. A further understanding on the role of epigenetic processes in cardiovascular disease processes such as atherosclerosis and restenosis might provide novel opportunities to understand disease pathology. This would provide the necessary knowledge platform for design of alternative treatment strategies, which are aimed at interfering in these epigenetic processes for the management of atherosclerosis and restenosis.
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Histone acetylation modifiers in vascular remodeling – new targets for therapy in CVD


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