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Emerging Epigenetic Therapy for Vascular Proliferative Diseases

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1. Introduction

Atherosclerosis and restenosis, complex pathologies of blood vessels, are multifactorial diseases triggered by the inflammatory response to injury of endothelium. Remodeling of the injured vessel, proliferation and migration of vascular smooth muscle cells (VSMC) and elaboration and accumulation of extracellular matrix proteins are main traits of these diseases (Dzau et al., 2002; Libby, 2002; Pons et al., 2009; Ranganna et al., 2006; Ross, 1995;). Despite the substantial progress in understanding the etiology and the clinical management of atherosclerosis and restenosis, they are still life threatening diseases. Precise reasons are not still fully transparent. Different cell types; distinct cellular pathways and processes; and multiple genes within each participating cell types that are vulnerable to both genetic and environmental risk factors participate in the pathogenesis of atherosclerosis and restenosis.
Recently, it is recognized that besides the genetic control epigenetic mechanisms regulate development and maintenance of organisms or their interaction with surrounding environment through the coordination of a set of reversible modifications that turn parts of the genome 'off' and 'on' at strategic times and at specific sites causing changes in gene expression with no changes in DNA sequences (Ekstrom, 2009; Pons et al., 2009; Ranganna et al., 2006; Turunen, 2009). The two well-known epigenetic mechanisms, DNA methylation and histone modifications change the chromatin structure and dynamics that alter gene functions by influencing gene expressions. Dysregulation of epigenetic processes has been linked to human diseases, which influences many aspects of cell biology including cell growth, cell cycle control, proliferation, differentiation, and cell death. Reversing the dysregulation of epigenetic mechanisms may offer effective treatment strategy for many diseases including cardiovascular disease due to atherosclerosis and restenosis. This review presents the current advancement in the epigenetics of VSMC proliferation and potential use of histone epigenetic modifiers in the intervention of atherosclerosis and restenosis.

2. Overview of pathogenesis of atherosclerosis

Atherosclerosis, a disease of medium to large arterial vessels, accounts for over 55% of all deaths in western countries. It is typically asymptomatic for decades but ultimately result in life-threatening pathological outcomes like myocardial infarction and stroke, both with tissue infarction because of intra-arterial thrombosis provoked by atherosclerosis.

Atherosclerosis is a complex progressive disease in which intimal thickening of the arterial wall promotes luminal stenosis by vascular remodeling, accumulation of cellular and extracellular substances and VSMC proliferation and migration. Integrity of arterial wall is crucial for the regulation of vascular tone, control inflammation, thrombosis, and angiogenesis, enhance regional blood flow, and inhibit cancer metastasis. Arterial wall is composed of three tunics that surround a central lumen through which blood flows. The innermost layer is the tunica intima composed of endothelial cells that form a smooth lining that minimizes interaction with circulating cellular and non-cellular components as blood moves through the vessel. The middle layer, tunic media, is composed of vascular smooth muscle cells (VSMC) and layers of flexible proteins, which enables the lumen to contract and dilate to regulate blood flow in the body. The outer layer, tunica adventitia is a protective layer of connective tissue that anchors the blood vessel to surrounding structures.

Under normal conditions, a delicate balance between proliferation and apoptosis of local vascular cell types maintains the thickness of arterial vessel wall. A number of regulatory factors produced by the endothelial cells are responsible for the homeostatic balance by controlling vessel tone, coagulation state, leukocyte trafficking, and cellular proliferative response. Any damage to the vessel wall by mechanical, biochemical, or immunological insults triggers endothelial dysfunction or denudation of endothelial layer overwhelming the normal homeostatic balance, thus, upsets the normal vascular tone setting the stage for the activation of proinflammatory and immune response. Escalating evidence indicates that inflammatory or atherogenic stimuli promote ROS generation in endothelial milieu causing oxidative stress (Freeman & Crapo, 1982; Kehrer, 1993; Kunsch & Medford, 1999; Madamanchi et al., 2005). Inflammatory response fueled by the oxidative stress is also linked to oxidation of lipoproteins. LDL molecules that enter the subendothelial space are oxidized to form oxidized LDL (OxLDL) by different mechanisms including enzymatic and nonenzymatic pathways, which are taken up by macrophages via scavenger receptors to become foam cells. Besides stimulating proinflammatory and proatherogenic effects, OxLDL also appears to elicit highly immunogenic response resulting in the generation of autoantibodies that appears to be of pathogenic significance (Hansson, 2009; Klingenberg, R., & Hansson, G.K. 2009; Steinberg & Witztum, 2010; Witztum, 1997). Moreover, elevated levels of ROS appear to function as second-messenger molecules transmitting the extracellular signals to nucleus via redox-sensitive signaling pathways to turn on the expression of atherogenic gene products such as adhesion molecules and inflammatory cytokines. Expression of these gene products elicit changes in the vessel wall promoting inflammation, infiltration of monocytes and T cells, proliferation, migration and activation of VSMC and matrix alteration (Freeman & Crapo, 1982; Hansson, 2007; Kehrer, 1993; Klingenberg & Hansson, 2009; Kunsch & Medford, 1999; Madamanchi et al., 2005; Steinberg & Witztum, 2010; Witztum, 1997). These processes involve synthesis and release of a host of regulatory molecules, both by cellular components in the blood and vascular cells of the arterial wall triggering autocrine, paracrine, and endocrine type of interactions between cells and the molecules they produce. Outcome of these complex interactions leads to migration of VSMCs from their normal residence in the arterial media to the intima where they change their phenotype from a contractile to a proliferative type (Libby, 2002; March et al., 1999; Ross, 1995). This phenotypic change, in conjunction with excessive production and accumulation of extracellular matrix proteins, is the main contributor to vascular remodeling.

2.1 Vascular remodeling in atherosclerosis and restenosis

Atherosclerosis and restenosis both are multifactorial vascular occlusive processes but exhibit certain similarities and differences in the origin and progression of their development (Dzau et al., 2002; Pons et al., 2009). Inflammatory response of activated endothelial cells to injury or insults elicits both these processes. Activation of endothelial cells leads to a cascade of events, which promotes vascular remodeling by changing the size, structure and composition of vessel wall. Moreover, both processes involve proliferation, migration and activation of VSMC and modulation of extracellular matrix by elaborating and accumulating extracellular matrix proteins. Although they share some of the risk factors such as hypertension and diabetes, there is a consensus that atherosclerosis develops in response to elevated low-density lipoproteins (LDL) and cigarette smoke. On the other hand, VSMC proliferation is the primary pathophysiological mechanism in restenosis, which is largely due to transcending wound healing response to clinical procedures such as balloon angioplasty, stent placement and vein graft surgery [Dzau et al., 2002; Pons et al., 2009]. While restenosis appears to be insensitive to circulating lipids, accumulation of oxidized LDL is the characteristic feature of atherosclerosis. Additionally, while development and progression of atherosclerosis is a gradual process, the restenotic process is a relatively a rapid process caused by surgical revascularization procedures such as angioplasty and stent placement. Despite substantial progress in understanding the etiology and the clinical management of atherosclerosis and restenosis, they are still life-threatening diseases. Possible reasons are multiple factors, different cellular pathways and processes, and multiple genes are contributory to these complex vascular disease processes.

2.2 VSMC proliferation

VSMC are highly specialized cells, which play vital roles in the regulation of blood pressure, blood flow and in many pathological states. In mature individuals, the typical function of arterial VSMC is contraction and maintenance of vascular tone. As such, VSMC in adult artery exhibit contractile or differentiated phenotype displaying quiescent proliferation state, decreased synthetic activity and expression of proteins unique to contractile phenotype like contractile proteins, ion channels and signaling proteins. However, VSMC retain their remarkable plasticity to undergo reversible phenotypic change in response to alterations in the local environment like during development, physiological conditions like pregnancy or in response to vascular injury. This remarkable flexible persona of VSMC makes them vulnerable to phenotypic modification from contractile to proliferative, synthetic, or de-differentiated phenotype in conjunction with vessel remodeling by altering cell number and composition of vessel wall (Pons et al., 2009; Ross, 1993).

VSMC proliferation is also the primary pathophysiological mechanism in different clinical pathologies such as postangioplasty restenosis, in-stent restenosis, vein bypass graft failure and transplant vasculopathy (Dzau et al., 2002; Holmes, 2003). The clinical procedures performed to clear the occluded vessel fortuitously become precursors for restenosis in 30-40% of the patients, mainly due to proliferation and activation of VSMC. While entry into cell cycle followed by proliferation of VSMC contributes to the formation of neointima, activation of VSMC induces expression of proinflammatory cytokines, adhesion molecules, chemoattractants, proteolytic enzymes and other molecules not usually present in normal, quiescent, contractile VSMC of the medial layer (Kleemann et al., 2008; Li et al., 1993; O'Brien et al., 1996; Zeffer et al., 2004). Expression of these molecules amplifies the

inflammatory response, and in turn, increases further proliferation of VSMC and elaboration of vessel remodeling.

2.3 Antiproliferative therapeutics to target VSMC proliferation

The current therapies used for atherosclerosis aim to minimize the risk factors that promote atherosclerosis, such as reducing elevated levels of cholesterol or enhancing the blood flow by surgical intervention of an already occluded vessel. Ironically, the surgical procedures performed to clear the occluded vessel become a precursor for restenosis, mainly due to VSMC proliferation, in significant number of patients (Dzau et al., 2002; Ferns et al., 1991). Because proliferation of VSMC is the hallmark of atherosclerosis and clinical conditions such as arterial stenosis, transplant vasculopathy, and bypass graft failure, the suitable therapeutic approach is to develop strategies that inhibit or block VSMC proliferation. Based on the current understanding of the molecular basis of vascular proliferative diseases, there is an abundance of potential therapeutic possibilities. Accordingly, a number of agents are tested for antiproliferative activity including heparins, cytostatic agents, inhibitors of angiotensin converting enzyme, and antagonists to growth factors (Dzau et al., 2002; Ferns et al., 1991; Gershlick, 2002; Stephen et al., 2005; Toshiro et al., 2005). Although some of these agents have shown promise in animal models, they failed to elicit any protection in human clinical studies (Gershlick, 2002). Species differences, potential toxicity, and lack of potency are possible culprits. Furthermore, the probability of successfully treating a multifactorial disease by targeting a single factor is unlikely. Additionally, all vascular cell types secrete growth factors and cytokines that activate signaling pathways that are redundant and thus prevent the success of targeting one or two factors.

2.4 Cell cycle as the therapeutic target

Based on the current knowledge of cell cycle mechanisms, it appears that targeting specific parts of the cell cycle is a better strategy to inhibit or block the development of vascular proliferative diseases such as arterial restenosis; in-stent-stenosis and vein bypass graft failure. Moreover, cell cycle is the final common pathway where all the growth regulatory signals converge, and thus, makes a rational target of antiproliferative therapeutics to inhibit vascular proliferative diseases. Some of the experimental studies indeed reveal that inhibition of cell cycle progression emerges as an important therapeutic target for prevention of vascular proliferative diseases (Dzau et al., 2002; Ranganna et al., 2006; Von der Leyen & Dzau, 2001). Different approaches such as pharmacological agents, irradiation, and gene therapy have been used for arresting VSMC proliferation. These approaches inhibit proliferation by cytostatic or cytotoxic mechanism. However, cytostatic mechanism of cell cycle arrest is desired over cytotoxic mechanism to avoid unintended damage to the vessel wall due to cytotoxic treatment. Three different approaches have been tried for arresting VSMC proliferation by targeting cell cycle, which include: 1) brachytherapy, 2) gene therapy, and 3) pharmacotherapy.

2.4.1. Brachytherapy

Endovascular radiotherapy is a promising method for effective antiproliferative treatment of restenosis (Teirstein & King, 2003; Waksman, 2000). Radiotherapy directed at restenosis has two objectives, one to treat restenosis by killing the cells that re-occluded and to prevent further restenosis by inhibiting tissue growth. Brachytherapy with either beta or gamma

radiation sources are used to diminish restenosis in patients with post-angioplasty restenosis or with in-stent restenosis. The rationale for using radiation for treating restenosis is that uncontrolled proliferation of VSMC is similar to neoplastic cells that can be targeted for radiation therapy just as transformed cells in cancer tissue. Brachytherapy-induced DNA damage of VSMC can result in arrest of VSMC at the G1 checkpoint or induction of apoptosis through p53 induced p21Cip1 upregulation. A key feature of brachytherapy is that the irradiation only affects a precise localized area around the radiation sources. Exposure to radiation of healthy tissues further away from the sources is therefore, reduced. In addition, brachytherapy is associated with a low risk of serious adverse side effects. More than a dozen randomized trials established its safety and efficacy. However, it exhibits two radiotherapy-related problems, arterial narrowing adjacent to the edge of the target site and unexpected late coronary thrombo-occlusive events (Raizner, 2000).

2.4.2 Gene therapy

Gene therapy techniques provide a unique opportunity to genetically engineer vessels and grafts to become impervious to atherosclerosis and neointimal formation that contributes to arterial restenosis, in-stent restenosis and vein graft failure (Dzau et al., 2002; Khanna, 2008; Kishore & Losordo, 2007; Gaffney et al., 2007; Melo et al., 2005; Von der Leyen & Dzau, 2001). Gene therapy approach has potential not only against monogenic diseases, but also against complex diseases where multiple genes are involved in the disease pathogenesis like in cardiovascular diseases and cancer. One of the key challenges of the gene therapy is appropriate vector for the delivery of functional gene or a concoction of genes in multigenic diseases as in cardiovascular diseases. Besides the choice of vector, other parameters such as, appropriate gene targets and efficient methods of vector delivery for a specific target have to be optimized. Vectors can be either viral or non-viral. The ideal vector is the one, which is nonpathogenic, less immunogenic, more efficient, and enhanced tissue specificity.

Delivery of therapeutic genes to the cardiovascular tissues is challenging. To facilitate local gene delivery to lesions in the vasculature catheter-based vector delivery has been tried using a variety of balloon catheters in animal models and human trials (Khanna, 2008; Kishore & Losordo, 2007; Gaffney et al., 2007; Melo et al., 2005). Stents are ideal gear for localized gene delivery to the vascular wall because of their widespread use, safety and permanent scaffold structure. Stents can be coated with genetically engineered cells or plasmid or adenoviral vectors carrying therapeutic genes (Khanna, 2008; Kishore & Losordo, 2007; Gaffney et al., 2007; Melo et al., 2005). Experimental studies have demonstrated usefulness of gene therapy in treating atherosclerosis and restenosis in various animal models and in some clinical trials. It can be used to transfer exogenous genes to express functional gene products to overcome defective or downregulated endogenous gene expressions through vector-based delivery system. It also can be used to knockdown or suppress the expression of gene products that contribute to pathogenesis of disease by one of the several methods of gene silencing. These include antisense oligonucleotides (ODNs), short segments of RNA with enzymatic activity (ribozymes) and small interfering RNAs [siRNA] (Dzau et al., 2002; J.M. Li et al., 2010; Banno, et al., 2006).

A number of studies have shown that gene therapies can be targeted for reducing cholesterol levels, inflammation and thrombosis (Feldman & Isner, 1995); for upregulating apo-A1 and downregulating chemoattractant protein-1 (MCP1)receptor expression (Tangirala, 1999); transferring pleiotropic atheroprotective nitric oxide synthase [NOS]

(Qian, 1999); targeting vascular redox biology through heme oxygenase-1, superoxide dismutase, catalase and glutathione peroxidase antioxidant gene therapy to attenuate oxidative stress (Van Assche, 2011); and lipid-lowering gene therapy to reduce plasma LDL levels (Grossman et al., 1995). Furthermore, neointimal hyperplasia that contributes to pathogenesis of arterial stenosis, in-stent stenosis and vein graft failure is also a good target for gene therapy. A number of potential therapeutic genes, which are key to the development of neointimal hyperplasia, have been identified. The ones that are promising for gene therapy include tissue inhibitors of matrix metalloproteinases (Akowuah et al., 2005; Gaffney et al., 2007; Khanna, 2008); NOS (Cooney, 2006; Dzau et al., 2002; Khanna, 2008; Kishore & Losordo, 2007; Gaffney et al., 2007; Melo et al., 2005; Von der Leyen & Dzau, 2001) and p53 (Gaffney et al., 2007). Importantly, delivery of antiproliferative genes such as those coding for p21Cip1, p27Kip1, and iNOS are used to inhibit stenosis and neointimal hyperplasia (Dzau et al., 2002; Von der Leyen & Dzau, 2001]. Conversely, silencing the genes that contribute to proliferation via antisense ODNs (Dzau et al., 2002; Khanna, 2008; Kishore & Losordo, 2007; Gaffney et al., 2007; Melo et al., 2005) or siRNA approach is also effective in preventing in-stent and graft neointimal hyperplasia (Banno, et al., 2006; F. Li et al., 2005; J.M. Li et al., 2010; Matsumae et al., 2008). Antisense ODNs–based inhibition of cell proliferation-related genes such as PCNA, c-myc, c-myb or different cyclin-dependent kinases (cdks) have been successfully carried out in experimental models of vascular lesion formation (Braun-Dullaeus et al., 1998; Dzau et al., 2002; Morishita et al., 1993; Simons et al., 1994).

RNA interference (RNAi) technology is becoming popular approach to alter gene expressions to interrogate their role in pathogenesis of disease, which has utility in the inhibition of VSMC proliferation and neointimal hyperplasia (Banno, et al., 2006; F. Li et al., 2005; J.M. Li et al., 2010; Matsumae et al., 2008). To determine whether Angiotensin II (ANG II)-induced neointimal thickening is mediated via cytoplasmic phospholipase A2 (cPLA2) - and phospholipase D2 (PLD2)-activated Akt, injured carotid arteries were exposed to a retrovirus containing $cPLA_2$ siRNA or PLD2 siRNA to test whether their knockdown will result in the reduction of ANG II-induced neointimal thickening (F. Li et al., 2005). SiRNA-mediated downregulation of cPLA2 and PLD2 resulted in the reduction of ANG II-induced neointimal thickening. The involvement of CCN1, an extracellular matrix-associated protein, in the development of neointimal hyperplasia is confirmed by siRNA-mediated knockdown approach (Matsumae et al., 2008). The atheroprotective role of midkine (MK), a heparin-binding growth factor, is corroborated by the use of MK-siRNA (Banno, et al., 2006). NADPH oxidase has a critical role in the development of neointimal hyperplasia and restenosis due to its contribution to oxidative stress, which is blocked by siRNA specific to NOX2 gene *Cybb,* an important component of NADPH oxidase (J.M. Li, et al., 2010).

Several experimental gene transfer and gene silencing strategies are evaluated as potential treatments for cardiovascular disease, which resulted in Phase I, and Phase I to Phase III clinical studies for inducible iNOS (Tzeng, 1996; Von der Leyen & Dzau, 2001) and transcription factor E2F, respectively (Dzau et al., 2002; McCarthy, 2001; Mann et al., 1999). E2F, a transcription factor that leads to upregulation of up to 12 cell-cycle genes is an ideal target for cell-cycle blockade. A double-stranded E2F decoy ODN that bears the consensus E2F-binding site (*cis*-elements) was designed as an agent for prevention of vein graft disease. In rabbits, treatment of vein grafts with E2F decoy ODN resulted in inhibition of neointimal hyperplasia and graft atherosclerosis for up to 6 months. This led to phase I

PREVENT trial for human vascular bypass grafts, which resulted in about 75% reduction in VSMC proliferation and fewer graft occlusions. Similar gene manipulation approach was used for coronary bypass grafts in PREVENT II trial. Although the phase 1 trial (PREVENT Trial) showed promising results, later studies were less positive including the phase III, multicentre, randomized double-blinded, placebo-controlled trial of 3014 patients undergoing primary coronary artery bypass graft surgery with at least two planned saphenous grafts (Alexander et al., 2005; Conte et al., 2005, 2006). Although appears to be promising, the use of gene therapy in the treatment of vascular proliferative diseases is still in infancy. Various feasibility and efficacy issues as well as design and delivery of the genes have to be addressed taking into account the complexity of the pathological processes leading to atherosclerosis and restenosis.

2.4.3 Pharmacotherapy

A number of pharmacological agents have been used to target injury-induced VSMC proliferation that contributes to neointimal growth in balloon-injured arteries. Among these rapamycin or sirolimus, a cytostatic agent, arrests VSMC proliferation and migration *in vitro* and reduces neointimal growth in animal models of balloon-injury (Dzau et al., 2002; Guerin et al., 2005). Its action appears to be mediated through the inhibition of mammalian target of rapamycin (mTOR). One of the downstream events induced by the inhibition of mTOR is induction of p27Kip1, an inhibitor of cyclin-dependent kinases (cdk), causes cells to arrest in G1 phase of the cell cycle. In doing so, it inhibits cell proliferation. Paclitaxel, a derivative of Taxol, is another promising agent for proliferation arrest, which by collapsing the mitotic spindle formation causes mitotic arrest (Jordan et al., 1993). It prevents neointima formation in animal models, and its clinical effect in the blockade of restenosis is investigated in several human trials (the ELUTES, TAXUS and ASPECT trials) via local delivery through stents coated with paclitaxel (Finn et al., 2007; Wilson et al., 2007).

2.4.4 Immunotherapy

Over the past several years, accumulating data have identified involvement of several antigens in the initiation of immune response during atherosclerosis. These include exogenous infectious microbial pathogens like, cytomegalovirus and chlamydia pneumonia and endogenous proteins such as oxLDL, heat shock proteins (HSPs) and β_2-glycoprotein-1b (Habets et al., 2010). Among these, the epitopes recognized on oxLDL are important because of the role of oxLDL in the pathogenesis of atherosclerosis ((Hansson, 1997; 2007; Steinberg & Witztum, 2010; Witztum, 1997). In addition to its proinflammatory and proatherogenic effects, and participation in the formation of foam cells, oxLDL is also immunogenic due to the presence of several neoepitopes. A number of neoepitopes generated during the oxidation of LDL are highly immunogenic and cause the generation of autoantibodies, which are detected in atherosclerotic lesions. Since the different epitopes of oxLDL induce atherogenic immune response, it may be possible to inhibit proatherogenic effects of oxLDL by modulating the immune response towards oxLDL through the immunization against oxLDL. Several antigens have been identified and investigated for immunization against atherosclerosis in animal models. Immunization against oxLDL show reduction in atherosclerosis in several animal models (Habets et al., 2010). This discovery of atheroprotective immunity has resulted in the emergence of immunotherapy approach against atherosclerosis. Indeed, several animal studies indicate that immunization against

oxLDL offers protection against atherosclerosis, which appears to operate both through cellular and humoral immunity (Zhou, 2001). The increased titers of T cell-dependent IgG antibodies to oxLDL (Habets et al., 2010; Zhou, 2001) and natural IgM antibodies to phosphocholine (Binder et al., 2004) are also in agreement with the atheroprotection. Furthermore, two recent studies report promising immunotherapeutic approach for the prevention of atherosclerosis. In one study, LDL-receptor deficient mice were vaccinated with oxLDL-pulsed mature dendritic cells to determine the effect on atherosclerosis (Habets et al., 2010). In the second immunotherapy study, tolerogenic apo-B100-loaded dendritic cells in combination with immunosuppressive cytokine interleukin-10 were injected intravenously to hypercholesterolemic mice. This immunotherapy significantly prevented atherosclerosis by reducing autoimmune response against LDL (Hermansson et al., 2011) Although, these studies are encouraging and promising from a clinical perspective to translate these promising outcomes to the clinics, antigens that can be easily manufactured under good manufacturing practice conditions and that have a reproducible quality are necessary. However, several clinical studies are currently underway to evaluate the therapeutic implications of immunotherapy.

3. Epigenetics and vascular proliferative diseases

Advancement in technological innovations during the past 25 years has resulted in far-reaching in-depth comprehension of the biology and the etiology of vascular diseases, and thus influencing the perception of the pathophysiology of vascular proliferative diseases like atherosclerosis and restenosis. In spite of the substantial understanding of the etiology and the clinical management of these vascular proliferative diseases, they are still life threatening diseases and reasons are not fully evident. Based on the recent finding of the role of epigenetics in human diseases, it is proper to expect that epigenetic mechanisms enforces an additional layer of gene regulation that alters chromatin structure, and dynamics in the pathogenesis of vascular proliferative diseases (Ekstrom, 2009; Pons et al., 2009; Ranganna et al., 2006; Turunen, 2009). Epigenetic mechanisms are essential for the functioning of genomes to regulate normal development and maintenance of organisms, and to facilitate their interaction with surrounding environment. Compilation of the past 10 to 20 years of studies has resulted in the identification of three highly interrelated epigenetic mechanisms that alter the chromatin structure and accessibility. These include, DNA methylation, histone posttranslational modifications and non-coding RNA (ncRNA) expression based mechanisms, each of these mechanisms is essential for regulation of gene expression. Therefore, it is anticipated that the genetic and environmental factors that are relevant to the development of vascular proliferative diseases by their effect on inflammation, VSMC proliferation and vessel remodeling, is regulated by epigenetic mechanisms through the modification of chromatin structure, dynamics and accessibility (Ekstrom, 2009; Pons et al., 2009; Ranganna et al., 2006; Turunen, 2009). Although there is an outbreak of interest and enthusiasm in linking altered epigenetic mechanisms to human pathologies particularly cancers, it is relatively unexplored area regarding cardiovascular diseases. Moreover, deregulation of epigenetic processes are linked to changes in many aspects of cell biology including cell growth, cell cycle control, and cell death by altering the expression and in turn functions of target genes without changing their primary gene structure. Because VSMC proliferation is the hallmark of vascular proliferative diseases, understanding the

epigenetics of VSMC proliferation and in particular their susceptibility to perturbation by the epigenetic modifiers may offer novel insights into disease pathogenesis and epigenetic therapeutic approaches. Therefore, it is appropriate to review the current knowledge of epigenetics in the regulation of VSMC proliferation.

3.1 VSMC epigenetics

Curiosity in epigenetics has surged during past decade even though the principle question it aims to address has been there for decades. That is, how a multicellular organism maintain drastically different gene expression profile in different cell types of the organism, while all the different cell types of the organism have exactly the same DNA. This is where epigenetics come into picture. Epigenetics refers to the inheritance of gene function/activity/expression that may be stable over long periods, last through several cell divisions or inherit through several generations, all without any change in their primary DNA (Ng & Gurdon, 2008; Probst, 2009). The three interrelated epigenetic mechanisms, which involve: methylation of DNA at CpG dinucleotides at specific position in the DNA molecule suppress expression of nearby genes (Esteller, 2008); posttranslational modifications of histones alters chromatin structure and changes promoter accessibility (Kouzarides, 2007); and small RNA molecules generated from non-coding RNAs (ncRNAs) inhibit gene expression (Mattick et al., 2009). All these mechanisms involved in epigenetic regulation contribute to epigenome. This review focuses on the role of posttranslational modifications of histones in the regulation of VSMC proliferation, and on the epigenetic regulators of histone modifications as potential candidates for drug targeting in the treatment and management of vascular proliferative disease.

3.2 Chromatin structure

Chromatin is a nucleoprotein complex consisting of repeating units of nucleosomal core particles. It offers a dynamic platform for all DNA-mediated processes within the nucleus. The nucleosomal core particles are the basic units of chromatin consisting of 147 base pair (bp) of DNA that wraps almost twice around two copies of each of the four core histone proteins, H3, H4, H2A and H2B. Each nucleosome is separated by 10-16 bp long linkers DNA, which gives an appearance of a bead on a string structure that constitutes the chromatin fiber of ~10 nm in diameter. The linker DNA assists further compaction of chromatin structure into higher-order chromatin structure, which is essential for packaging of remarkable lengths of DNA into the cell nucleus. Furthermore, this compact chromatin structure limits accessibility of DNA to DNA-mediated processes like transcription, DNA replication, and DNA repair (Kouzarides, 2007). Evidence accumulated during the past 15 years reveals that three interrelated epigenetic mechanisms alter the highly compacted chromatin structure and facilitate accessibility of DNA for gene transcription. The interrelated epigenetic mechanisms that include DNA methylation, histone modification and ncRNA expression contribute to the epigenome making the epigenome dynamic rather than static like genome and thus, being predisposed to and influenced by environmental factors and extracellular stimuli. Deregulation of epigenetic mechanisms is observed in many different cancers and other human diseases. Thus, understanding of how epigenetic mechanisms contribute to gene regulation will provide insight into the disease process.

3.3 Histone modifications

Histones are highly conserved basic proteins that undergo an amazing number and types of posttranslational modifications, which contributes to the active or inactive chromatin (Ekstrom, 2009; Kouzarides, 2007; Pons et al., 2009; Ranganna et al., 2006; Turunen, 2009). Each of the four core histones are composed of a conserved globular domain that forms the nucleosome core, and a highly dynamic amino-terminal tail of 20-35 residues rich in basic amino acids. Additionally, H2A histone has an extended tail of about 35 residues at the carboxy-terminal end. Both amino- and carboxy-terminal tails protrude from nucleosome into the nucleoplasm. Histones tails are the targets of an array of site-specific posttranslational modifications including lysine acetylation/deacetylation, lysine and arginine methylation, serine and threonine phosphorylation, lysine ubiquitylation and sumoylation, and glutamic acid ADPribosylation (Fischle et al., 2003; Ito, 2007; Jenuwein & Allis, 2001; Turner, 2003).

Histone modifications are dynamic and reversible, and their 'off' and 'on" modification states are influenced by different physiological and environmental factors like developmental state, stress condition and environmental cues. Many of histone modifications are associated with transcriptionally active euchromatin regions, while other histone modifications are localized to transcriptionally inactive heterochromatin regions. Even though issues such as how the process of modification is regulated, and how many modifications are required for their biological effect are still elusive, recognition of specific histone modifications by various effector proteins is suggested to mediate specific biological processes like gene activation or gene suppression by altering the chromatin structure and gene accessibility. Generally, conformationally relaxed and decondensed chromatin structure that is associated with histone acetylation and DNA hypomethylation is the feature of transcriptionally active chromatin. On the other hand, compact and condensed chromatin structure that is associated with deacetylation of histones and hypermethylation of DNA is transcriptionally silent. Condensed chromatin structure is essential during cell cycle, mitosis and meiosis, whereas decondensed chromatin structure is required for gene expression, replication, repair and recombination. The combinatorial pattern of the histone modifications indicates the state of the chromatin structure, and thus, regulates the accessibility of the DNA to the transcription-regulatory complexes, through it controls gene expression. The collection of various covalent histone modifications serve as epigenetic marks for the recruitment of different proteins or protein complexes to regulate disparate chromatin functions such as gene expression, gene suppression, mitosis, repair, replication and chromosome segregation (Taverna et al., 2007). Furthermore, there is also crosstalk between different histone modifications like acetylation, methylation and phosphorylation at independent sites forming a "histone code" that is translated to a specific biological event through the mediation of various effector proteins. For example, phosphorylation of serine 10 of histone H3 facilitates acetylation of lysine 14 and methylation of lysine 4, which create an open or relaxed chromatin conformation associated with an active gene. Serine 10-phosphorylation also facilitates the acetylation of lysine 9, thus preventing the repressive lysine 9-methylation associated with an inactive gene (Jenuwein & Allis., 2001; Lund & van Lohuizen, 2004; Mathew et al., 2010; Schreiber & Bernstein, 2002; Turner, 2003).

3.4 Chromatin modifying enzymes

Consistent with the variety of posttranslational modifications of histones, a disparate family of enzymes, which are referred as histone modification writers, catalyzes addition of specific functional groups to histones. These modifications of histones alter the chromatin structure and function by two distinct manner: First, by directly altering the charges of histone proteins, certain posttranslational modifications cause localized relaxation of chromatin structure and second, by serving as recognition and binding sites for various classes of effector proteins that participate in chromatin remodeling, certain histone modifications indirectly alter the chromatin structure. However, most histone modification are reversible and diverse families of proteins, which include histone acetyltransferases (HATs) /histone deacetylases (HDACs), histone methyltransferases (HMTs)/demethylases, histone kinases/phosphatases, and ubiquitin ligases, catalyze addition and removal of the modifications from histones. One of the highlights of epigenetics is that it offers new therapeutic targets for diseases including cardiovascular diseases. The epigenetic regulators, HATs/HDACs and HMTs/demethylases, which exhibit counterbalancing activities are essential for the regulation of gene expression, which are required for the basic cellular processes such as cell proliferation and differentiation. This essential role of epigenetic regulators in basic cellular processes identifies potential therapeutic targets for diseases including cardiovascular diseases. Moreover, identification of those HATs and HDACs that plays a role in the transcriptional regulation of genes, products of which contributes to the processes of neointima formation like inflammation, VSMC proliferation, and matrix formation is also important in designing potential epigenetic therapy to target vascular proliferative diseases. Thus, pharmacological inhibition of enzyme activities involved in epigenetic DNA and histone modifications designed to induce or silence the transcription of disease-relevant genes offers an amenable therapeutic intervention for atherosclerosis and restenosis. In addition to modifying the effects of diseased genes, it is possible to change the effects of environmental risk factors by targeting epigenetic mechanisms. Here we will focus on HATs/HDACs, the principal epigenetic regulators that control histone acetylation, a major epigenetic modification for transcriptional control of gene expression. Because HATs/HDACs are essential for the regulation of gene expression, in all probability, they play crucial role in the development of multigene and multifactorial diseases such as atherosclerosis and restenosis.

4. Histone acetyltransferases (HATs) and histone deacetylases (HDACs)

One of the best- and most-studied posttranslational histone modifications is lysine acetylation catalyzed by HATs, the modification that is generally associated with gene activation (Ekstrom, 2009; Kouzarides, 2007; Pons et al., 2009; Ranganna et al., 2006; Turunen, 2009). Hyperacetylation of histones causes decondensation of chromatin allowing a more relaxed or open and active chromatin structure, which allows accessibility of DNA to basal transcription initiation machinery (Kouzarides, 2007; Roth, 2001). In contrast, gene repression is mediated by HDACs, and other co-repressors, which cause deacetylation of hyperacetylated histones and offset the activity of HATs resulting in a closed conformation of chromatin structure. Thus, the acetylation status of the chromatin associated with particular genes is dictated by the balance between the activities of HATs and HDACs. These enzymes are shown to regulate expression of genes associated with various cellular processes like inflammation, proliferation and matrix modulation (Cao et al., 2005; Pons et

al., 2009; Sahar et al., 2007; Vinh et al., 2008; Waltregny et al., 2005; Xu et al., 2007; Yan et al., 2009). HATs and HDACs are also recruited to gene promoters by multiprotein transcriptional complexes, where they regulate transcription through chromatin modification without directly binding the DNA.

A number of different HATs are identified and organized as families based on the presence of highly conserved structural motifs, which include PCAF/Gcn5, p300/CBP, MYST, SRC, and $TAF_{II}250$ families. While they all differ in their HAT domains and substrate specificity, they all require the assembly of multiprotein complexes for acetylation of nucleosomes (Marmorstein, 2001). Likewise a number of HDACs are identified and are classified into three different classes based on cellular localization, substrates and binding site features (Lindemann et al., 2004; Santini et al., 2007). Class I and class II include zinc-dependent HDACs, and class III includes NAD-dependent HDACs, which are also called as sirtuins. Class I HDACs are widely expressed and include HDACs 1-3 and 8 that are exclusively localized to nucleus. They are known to modulate cell proliferation and survival. Class II HDACs are HDACs 4-7, 9, and 10, which shuttle between the nucleus and cytoplasm in response to certain cellular signals. They may be involved in cell differentiation (Pons et al., 2009; Santini et al., 2007). Class II HDACs are further divided into Class IIa and Class IIb , which include HDACs 4, 5 ,7, and 9, and, HDACs 6 and 10, respectively. While Class IIa members have an extended N-terminal regulatory domain, Class IIb exhibit an extra catalytic domain (Lindemann et al., 2004; Pons et al., 2009; Santini et al., 2007). Class III HDACs are sirtuins (SIRT), which include NAD^+-dependent enzymes (SIRT 1-7) potentially involved in apoptosis (Pons et al., 2009).

HATs and HDACs are also recruited to gene promoters by multiprotein transcriptional complexes. There they regulate transcription through chromatin modification without directly binding the DNA (Johnstone & Licht, 2003; Pons et al., 2009). Moreover, HATs and HDACs are also involved in the acetylation status of lysine residues of transcription factors such as p53, E2F1, GATA1, RelA, YY1 and hormone receptors. Acetylation status of these transcription factors affects their DNA binding and transcriptional activity (Glozak et al., 2005; Johnstone & Licht., 2003; Marks, 2001; Pons et al., 2009). Besides histones and transcription factors, several other non-histone proteins like α-tubulin, nuclear import protein importin-α7, and signal transduction protein β-catenin are also modified by HATs and HDACs, but their effects on gene expression is not dependent on chromatin remodeling (Johnstone & Licht., 2003; Marks, 2001).

4.1 Epigenetic therapy targeting VSMC proliferation

Because HATs/HDACs are involved in dynamic reversible epigenetic processes that contribute to modulation of gene expression profiles specific to cellular processes like cell proliferation, they probably play important role in cardiovascular pathologies such as atherosclerosis and restenosis. Moreover, epigenetic deregulation affects several aspects of cell biology, including cell growth, cell cycle control, differentiation, DNA repair, and cell death. This elevates the strong possibility that reversing deregulated epigenetic mechanisms may be an effective treatment strategy for proliferative diseases. Incidentally, the property of HDACs, suppression of gene expression by epigenetic mechanism, has been exploited in the field of cancer to reactivate transcriptionally silent tumor suppressor gene to arrest proliferation of cancer cells and growth (Pons et al., 2009; Ranganna, et al., 2005, 2007; Sharma et al., 2010). Moreover, HDAC inhibitors (HDACi) are emerging as a new class of

anticancer agents that are under clinical trials for different cancer treatment. Some of the early clinical studies have demonstrated that certain HDACi exhibit promising activity against several neoplasms (Bhalla, 2005). Naturally, it has stimulated great interest to determine how HATs/HDACs regulate transcriptome of different processes that are linked to the development of atherosclerosis and restenosis like inflammation, VSMC proliferation and matrix modification and to assess therapeutic potential of HDACi in these vascular proliferative diseases (Ekstrom, 2009; Pons et al., 2009; Ranganna et al., 2006; Turunen, 2009). The following sections will focus on the role of HATs and HDACs in the transcriptional regulation of genes in the context of their contribution to VSMC proliferation and its disorders as well as on the potential applicability of HDACi in vascular disease management.

4.1.1 Histone deacetylase inhibitors (HDACi)

In the past few years, great effort has been focused on seeking and designing most effective HDACi because of their potential roles in reversing the silenced genes in tumor cells by modulating transcriptional processes. The balance between the acetylated/deacetylated states of histones, which is mediated by the counterbalancing activities of HATs and HDACs, contributes to the transcriptional states of chromatin structure. The structural modification of histones by acetylation/deacetylation of their N-terminal tails is crucial in modulating gene expression, because it affects the accessibility of DNA for the transcription-regulatory protein complexes. HATs preferentially acetylate specific lysine residues of histones, which relaxes the DNA conformation, thus allowing its access to transcription machinery to turn on gene expression. On the contrary, HDACs restore the positive charge on lysine residues by removing acetyl groups, which promotes condensed chromatin structure. This promotes silencing of gene expression by blocking the access of transcription machinery to DNA. Inappropriate silencing of critical genes such as tumor suppressor genes can result in cancer based on the recent understanding of the cancer cell cycle (Kristeleit et al., 2004). This provides a rationale for using inhibition of HDAC activity to release transcriptional repression. As result, a flurry of HDACi has been recognized for their ability to inhibit HDACs activity.

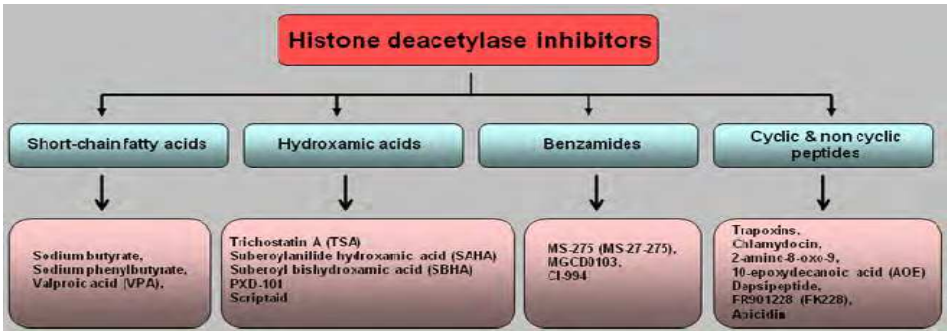

Fig. 1. Structural class of histone deacetylase inhibitors

Structurally diverse classes of naturally occurring and synthetic compounds have been recognized for their ability to bind to the catalytic pocket of HDACs and chelate the zinc ion

at its base, thereby inhibiting HDAC activity (Marks et al., 2000). A wide range of structures inhibits activity of class I/II HDAC enzymes with a few exceptions [Figure 1]. The HDACi are classified into structural classes including 1) short-chain fatty acids (carboxylates), 2) hydroxamic acids, 3) benzamides, and 4) cyclic and non-cyclic peptides. The various HDACi studied so far have been shown to inhibit class I (HDACs 1, 2, 3, and 8) and II (HDACs 4, 5, 6, 9, and 10) HDACs. Their activities have been tested in cell lines and preclinical murine models, and appropriate drugs that are selected for clinical trials, demonstrated good tolerance and clinical activity against different human neoplasms (Santini et al., 2007). However, Class III HDACs (SIRT 1, 2, 3, 4, 5, 6, and 7), also known as sirtuins, require NAD rather than zinc as a co-factor for their activity, and are not inhibited by the HDACi. Instead, they are inhibited by Nicotinamide (Luo et al., 2001).

4.1.2 HDACi effects on cellular processes

HDACi exhibit multiple cellular effects, which are linked to chromatin-mediated altered transcriptional activity. In general, most HDACi exhibit inhibition of cell proliferation, stimulation of cell differentiation and/or induction of cell death by selectively modulating gene expression (Bhalla, 2005; Mathew et al., 2010; Ranganna, 2005). HDACi arrest cells at the G1 or G2/M, and promote cell differentiation mainly by stimulating cyclin-dependent kinase inhibitor (cdkI) p21Cip1 expression (Bhalla, 2005; Mathew et al., 2010; Ranganna et al., 2005). HDACi also cause cell cycle blockade through the modulation of mechanisms that involve repression of cyclin D and cyclin A and upregulation of other cdkI like p27Kip1, p16INK4A and p15INK4B, which blocks pRb/E2F pathway, thus preventing the cell cycle progression (Mathew et al., 2010; Bhalla, 2005) [Figure 2]. Now with the array technologies, it is recognized that HDACi selectively modulate about 2% to 10% of all genes, with as many genes upregulated as are downregulated genes in different cell types (Bhalla, 2005; Ranganna et al., 2003). One of the genes that are universally upregulated is the cdkI p21Cip1, in a p53-independent manner, which is necessary for HDACi-induced G1 arrest. Induction of GADD45α and β and upregulation of transforming growth factor beta, which inhibits c-*myc* expression may also contribute to the cell cycle arrest in G1 or G2 (Bhalla, 2005.). HDACi treatment is also shown to transcriptionally downregulate the expression of CTP synthetase and thymidylate synthetase, which are required for DNA synthesis, thus, causing inhibition of S phase progression [Figure 3].

HDACi also stimulate differentiation of several cancer cells by inhibiting cell proliferation (Bhalla, 2005). Again, upregulation of p21Cip1 appears to be essential for differentiation because cells lacking p21Cip1 fail to respond to HDACi treatment (Bhalla, 2005). Furthermore, acute promyelocytic leukemic cells and primary leukemia blasts, expressed differentiated phenotype in response to a combination of ATRA, a retinoid-based chemotherapeutic drug and HDACi (Bhalla, 2005). HDACi stimulated gelsolin, an actin-binding protein required for morphological and cytostructural changes associated with differentiation (Bhalla, 2005).

It is interesting that HDACi induce growth arrest and cell differentiation in some cell, and in others, they cause apoptosis (Bhalla, 2005; Johnstone & Licht, 2003). HDACi-induced apoptosis triggers both the intrinsic and extrinsic pathways of apoptosis. Several types of HDACi, particularly hydroxamic acid analogs are shown to induce mitochondrial permeability transition, which releases prodeath molecules such as cytochrome c, Smac and Omi into cytosol (Bhalla, 2005). This triggers activation of Apaf-1, which leads to the

processing and activation of caspases-9 and-3 (Bhalla, 2005). HDACi appear to promote apoptotic cell death not only by upregulating several proteins that participate in apoptotic cell death including Bak, Bax, Bim, DR4, DR5, and TRAIL, but also by attenuating the levels of a number of antiapoptotic proteins such as Bcl-xL, Bcl-2, XIAP, and survivin (Bhalla, 2005).

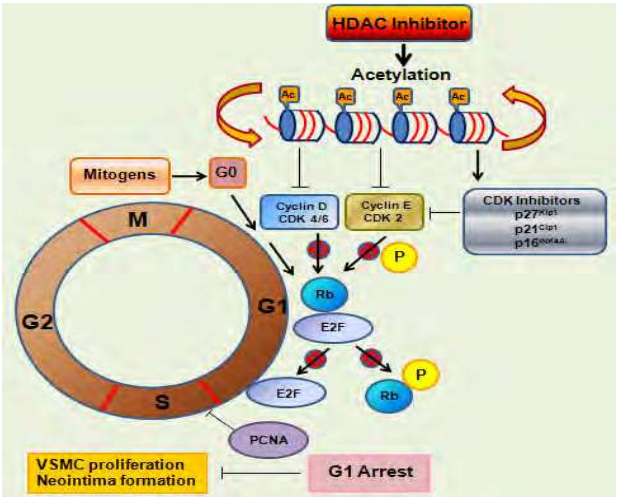

Fig. 2. Display of cell cycle targets of HDAC inhibition.

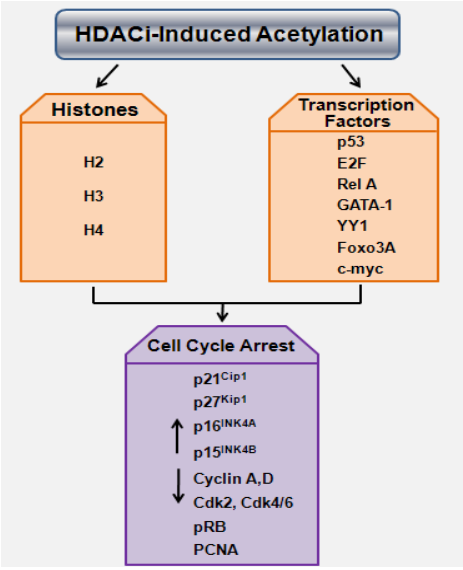

Fig. 3. Cell cycle regulatory proteins that are altered by the HDACi-induced acetylation of histones and transcription factors.

Besides affecting cell proliferation, differentiation and apoptosis, HDACi also alter the function of some of the non-histone transcription factors like p53, RelA, GATA1 and FoxO3A because HDACi enhance their acetylation, which may affect their DNA binding and transcriptional activity (Marks et al, 2001; Lindemann et al., 2004). Similarly, stimulating acetylation status of other non-histone protein such as nuclear import protein importin-α7, signaling protein β-catenin, DNA repair enzyme Ku70, and the cytoskeletal protein β-tubulin, HDACi alter their activity (Bhalla, 2005). Taken together, by inducing acetylation of histones and non-histones, HDACi alter the levels of proteins that control cell cycle progression, differentiation and apoptosis appropriately by transcriptional and post-transcriptional mechanisms, which implicate their potential use in disease treatment.

4.2 VSMC proliferation and histone acetylation

Although the common mechanism of pathogenesis shared by the atherosclerosis and cancer are linked to abnormal cell proliferation, very limited information is available with reference to anti-atherogenic potential of HDACi. Because HDACi not only alter gene expressions, but also cause inhibition of cell proliferation and induction of differentiation and/or apoptosis, a number of studies are initiated in the past few years to test the effects of HDACi as potential antiatherogenic agents. Even though both in vitro and in vivo studies have been done with the intention of targeting VSMC proliferation for the intervention and management of vascular proliferative diseases, most of the information that is available currently is from in vitro cell culture studies. There is limited in vivo data supporting the protective role of HDACi but needs further evaluation in models of VSMC injury.

4.2.1 VSMC proliferation

In general, HDACi exhibit almost same effects in VSMC as they do in cancer cells. They arrest cell proliferation, induce differentiation and/or apoptosis, and modulate expression of cell cycle regulators. Several studies have shown that trichostatin A (TSA), a well-known HDACi, arrests VSMC proliferation via upregulation of p21Cip1 and subsequent reduction of the phosphorylation of Rb protein at the G1-S phase (Okamoto et al., 2006; Pons et al., 2009), the effects consistently observed in cancer cell (Bhalla, 2005). In contrast, in one of the studies TSA unexpectedly exhibited paradoxical pro-atherogenic effect on VSMCs via the reduction of thioredoxin 1 instead of antiatherogenic properties (Song et al., 2010).

Besides TSA, butyrate, a well-known dietary HDACi, which has been used in different human cancer and other disease treatments, appears to exhibit potential antiatherogenic effect by arresting VSMC proliferation and appropriately altering both negative and positive cell cycle regulators (Davie, 2003; Mathew et al., 2010; Ranganna et al, 2005). Butyrate belongs to the class of short-chain fatty acids and is a derived from the intestinal microbial fermentation of dietary fiber. A number of epidemiological, animal and interventional studies suggest an inverse relationship between dietary fiber and chronic diseases such as bowel disorders and colorectal cancer, cancer of other tissues, cardiovascular disease, diabetes, obesity and hypertension (Anderson, 2003; Dashwood et al., 2006; Kim, 2000; Ranganna et al., 2005, 2006). Some of the studies suggest that the protective effect of dietary fibers in chronic diseases is linked to bioactivity of butyrate (Anderson, 2003; Dashwood et al., 2006; Kim, 2000; Ranganna et al., 2005, 2006). Butyrate elicits many cytoprotective, chemopreventive and chemotherapeutic activities mainly through inhibition of cell proliferation, stimulation of cell differentiation and/or induction of cell death by selectively

modulating certain gene expressions, but the mechanistic basis for these actions are far from clear. Butyrate has been known to alter chromatin structure and organization via hyperacetylation of histone amino-terminal tails, modulate gene expression and play a protective role in the prevention of cancer and inflammatory diseases of colon for a long time (Ranganna et al., 2005, 2006). However, its importance in the prevention of cancer of other tissues and different diseases has been recognized during the past ten years (Anderson, 2003; Dashwood et al., 2006; Kim, 2000; Ranganna et al., 2005, 2006). On the other hand, no similar studies are performed to indicate the protective role of butyrate in cardiovascular diseases due to atherosclerosis and restenosis.

During last few years, significant interest is focused on potential utility of butyrate and its stable derivatives in the intervention of vascular proliferative diseases, besides their therapeutic applications in other diseases including cancers. Butyrate and its more stable in vivo analogue tributyrin, arrested proliferation and inhibited DNA synthesis of smooth muscle cells in a cAMP-independent manner. Butyrate also abolished serum-induced c-fos, c-myc, and Ki-Ras expression that are important for early G1 events initiated by serum growth factors, but stimulated the expression of PS4 and thromospondin (Feng et al., 1996). Moreover, studies performed in our own lab further supports the efficacy of butyrate and its stable derivatives in vascular proliferative diseases. Treatment of VSMC with butyrate inhibited serum and PDGF-induced proliferation and abolished expression of proliferation markers such as c-myc and proliferating cell nuclear antigen [PCNA] (Ranganna et al., 1995, 2000). Furthermore, our analysis of profiles of VSMC transcriptome by array technology disclosed that butyrate-arrested VSMC proliferation is a multigene and multipathway-mediated process. Our array data identified differential expression of several genes in butyrate arrested VSMC proliferation, which are mainly belonging to four different functional classes: cell proliferation and differentiation; stress response; vascular function; and genes normally present in neuronal cells (Ranganna, et al., 2003). Extension of this study reveals that an upper level regulatory mechanism mediated through epigenetic modification of chromatin structure controls the expression of both positive and negative cell cycle regulatory genes linked to VSMC proliferation arrest by butyrate (Mathew et al., 2010). To establish the mechanistic link between chromatin remodeling and antiproliferation action of butyrate, influence of butyrate on posttranslational modifications of histone H3 and its consequence on G1-specific cell cycle regulators were investigated [Figure 2]. Outcomes of the study indicate interplay between different site-specific posttranslational modifications of histone H3 in butyrate treated VSMCs that seem to alter chromatin structure and organization that supports downregulation of cdk2, cdk4/6, and PCNA, and upregulation of cdkI, p21Cip1 and p15INK4B. This causes inhibition of Rb phosphorylation resulting in arrest of VSMC proliferation [Figure 2 and Figure 3]. The effects of HDACi on cell cycle-related gene expressions appear to be highly selective, leading to transcriptional activation of certain genes such as the cdkIs but repression of others like cdks to efficiently block cell proliferation.

4.2.2 Histone acetylation

Hypernuclear acetylation (HNA) also plays a role in proliferation (Kawahara et al., 2003). Presence of increased histone acetylation is observed in VSMC of atherosclerotic lesions unlike in normal arteries. Thrombin, a humoral factor that is known to activate and stimulate VSMC proliferation, strongly induced HNA in cultures of VSMC. MAP kinase

pathway and CBP are implicated in thrombin-induced HNA suggesting that coactivators cooperating with signaling-dependent transcription activators play a role in atherosclerosis through HNA (Kawahara et al., 1999).

5. Conclusions and perspectives

Over the past few years, it has become abundantly evident that several interdependent epigenetic changes collaborate with genetic changes in the development of human diseases including cardiovascular diseases such as atherosclerosis and restenosis. Since the genetic foundations of diseases are generally immutable, but their epigenetic and chromatin changes are reversible, they are suitable for epigenetic therapy with epigenetic and chromatin modifiers. Therefore, thorough understanding on the roles of epigenetic processes in the etiology of atherosclerosis and restenosis is essential to launch an epigenetic therapy designed to target the epigenetic processes. Although a number of different therapeutic approaches have been investigated in the treatment of atherosclerosis and restenosis such as brachytherapy, pharmacotherapy, gene therapy, and immunotherapy, the therapeutic efficacy of these treatment modalities for atherosclerosis and restenosis is not adequate for a number of patients. Possible reasons are multiple factors, genes, pathways are involved in the disease pathogenesis, and targeting one or two genes or pathways are not sufficient to treat complex vascular pathologies. In these scenarios, epigenetic therapy, which is reversible, appears to be appropriate because HDACi exhibit multiple cellular effects that play major roles in vascular pathogenesis. HDACi exhibit antiproliferative, antioxidant and antiinflammatory effects and cause inhibition of cell proliferation and stimulation of differentiation or apoptosis by modulating expression of multiple genes (Natarajan, 2011; Ranganna, et al.,2005; 2006; 2007). For example, HDACi inhibit cell proliferation by appropriately altering both positive and negative regulators of cell cycle. While cell cycle inhibitors such as p21Cip1, p27kip1, p16INK4a and p15INK4b are upregulated, expressions of cyclin D, cyclin A, cdk2, cdk4/6, PCNA and pRb that promote cell cycle progression are downregulated (Figure 2 and Figure 3). With one single HDACi, multiple genes are altered that control cell cycle progression unlike gene therapy, where a cocktail of genes is required to bring about inhibition of cell proliferation. Furthermore, stents are ideal platform for the localized delivery of HDACi to the vascular wall because of their widespread use and safety in the treatment of restenosis. It is recognized that many of the processes that play critical role in atherosclerosis and restenosis such as VSMC proliferation, migration, inflammation, cellular redox state and matrix protein synthesis (Natarajan, 2011) are regulated by epigenetic mechanisms. As such, they present an exciting opportunity for therapeutic intervention, particularly to refractory or recurrent vascular pathologies such as restenosis and in-stent restenosis and vein graft failure. A number of natural and synthetic HDACi are already in the pipeline for the treatment of cancer either stand alone, or in combination with other anticancer drugs and several clinical trials are in progress. Exploring these particulars will speed the necessary epigenetic treatment strategies for the management of atherosclerosis and restenosis.

6. Acknowledgements

The work from our laboratory described in this review is supported by G12RR0345 and C06RR012537-01 grants from National Institutes of Health/National Center for Research Resources.

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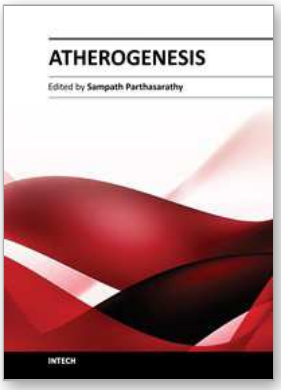

Atherogenesis

Edited by Prof. Sampath Parthasarathy

ISBN 978-953-307-992-9
Hard cover, 570 pages
Publisher InTech
Published online 11, January, 2012
Published in print edition January, 2012

This monograph will bring out the state-of-the-art advances in the dynamics of cholesterol transport and will address several important issues that pertain to oxidative stress and inflammation. The book is divided into three major sections. The book will offer insights into the roles of specific cytokines, inflammation, and oxidative stress in atherosclerosis and is intended for new researchers who are curious about atherosclerosis as well as for established senior researchers and clinicians who would be interested in novel findings that may link various aspects of the disease.

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