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Invited Review

Transcriptional regulation of endothelial dysfunction in atherosclerosis: an epigenetic perspective

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Abstract

Atherosclerosis is a progressive human pathology that encompasses several stages of development. Endothelial dysfunction represents an early sign of lesion within the vasculature. A number of risk factors for atherosclerosis, including hyperlipidemia, diabetes, and hypertension, target the vascular endothelium by re-programming its transcriptome. These profound alterations taking place on the chromatin rely on the interplay between sequence specific transcription factors and the epigenetic machinery. The epigenetic machinery, in turn, tailor individual transcription events key to atherogenesis to intrinsic and extrinsic insults dictating the development of atherosclerotic lesions. This review summarizes our current understanding of the involvement of the epigenetic machinery in endothelial injury during atherogenesis.

Keywords: Atherosclerosis, transcriptional regulation, endothelial injury, epigenetics

INTRODUCTION

Atherosclerosis represents a major factor of coronary heart disease characterized by the formation of fat-laden plaque in large and medium-sized vessels. During atherogenesis, the endothelial layer of the vessels is constantly confronted with a range of stress signals. Elevated levels of circulating oxidized low–density lipoprotein (oxLDL)[1], increased turbulent blood flow[2], and excessive inflammation[3] all contribute to endothelial injury. Regardless of the nature of the stress cue, the transcriptome of vascular endothelial cells is profoundly altered[4-8]. For instance, down-regulation of eNOS transcription and simultaneous up-regulation of ET-1 transcription in endothelial cells precedes the impairment of vasodilation and rhythmic vessel tone[9]. Transcriptional activation of adhesion molecules, on the other hand, enables circulating leukocytes to attach to the endothelium and establish a pro-inflammatory microenvironment[10]. Therefore, elucidation of the mechanisms underlying these characteristic transcriptional events will potentially further our understanding of atherogenesis and yield druggable targets for the intervention and prevention of atherosclerosis. Recent advances in the transcriptional regulation of atherosclerosis suggest a growing involvement of the epigenetic machinery[11,12]. This mini-review is a modest attempt to summarize the current state of research on the epigenetic regulation of endothelial disorder in atherosclerosis and to provide an outlook.

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EPIGENETIC REGULATION OF TRANSCRIPTION

Unlike prokaryotic organisms, eukaryotic transcription takes place on nucleosome-wrapped chromatin. In order for the basic transcription machinery to be recruited to promoter region and initiate transcription, chromatin has to be unwound to expose the binding sites. The epigenetic machinery, composed of histone modifying enzymes, DNA modifying enzymes, chromatin remodeling proteins, non-coding regulatory small RNAs, and histone variants, serves as an intricate regulatory layer for eukaryotic transcription by altering chromatin structure[13]. Due to space constraints, we only give a brief overview of histone modifications and chromatin remodeling, which will be the focus of discussion in this review.

Histone modifications

The N-terminal tails of core histones, H3 and H4 in particular, can be post-translationally modified. The term "histone code" was coined to correlate a specific set of histone modifications to a predictable transcriptional outcome[14]. Though a subject of constant controversy and debate, it is generally believed that acetylation of histones H3 and H4 surrounding the promoter region is synonymous with transcriptional activation. Whereas methylation of histone H3 lysine 4 (H3K4) may herald activation, H3K9 methylation often marks repressed chromatin[15].

Chromatin remodeling

In order for sequence-specific transcription factors and the basic transcription machinery to access the DNA and initiate transcription, the chromatin has to be unwound and loosened. This is achieved by moving the nucleosomes along the DNA at the expense of ATP hydrolysis[16]. Initially identified and characterized in yeast[17,18], the chromatin remodeling proteins represent a most conserved branch of the epigenetic machinery during evolution. The mammalian chromatin remodeling complex is a multi-subunit mega-protein conglomerate that contains a catalytic component. Brahma related gene 1 (Brg1) and Brahma (Brm) are the best studied chromatin remodeling proteins with ATPase activity[19]. Brg1 and Brm have been known to participate in both transcriptional activation and repression depending on the specific chromatin environment and the transcription factors they interact with[20].

EPIGENETIC REGULATION OF INDUCTION OF ADHESION MOLECULES DURING ATHEROGENESIS

Under physiological conditions, the vessel wall is free from the attachment of circulating leukocytes. Under certain pro-atherogenic conditions, such as turbulent shear stress, oxidative stress, intermittent hypoxia, and excessive nutrition, endothelial cells up-regulate the transcription of adhesion molecules (CAM) including ICAMs, VCAMs, and selectins, which consequently allow a much stronger interaction between leukocytes and the endothelium perpetuating a pro-inflammatory niche[10,21].

Diabetes is one of the leading causes for vasculopathies including atherosclerosis[22]. A seminal study by Brownlee and colleagues examined the effect of transient hyperglycemia on endothelial function[23]. Of great intrigue, these authors have found that exposure to high glucose (HG) for a short period time (16 hours) induced a prolonged activation of VCAM-1 in bovine aortic endothelial cells even when these cells were switched to and maintained in low glucose (LG) for additional 6 days, a phenomenon dubbed as "metabolic memory". Chromatin immunoprecipitation (ChIP) revealed an uptick of H4K4 monomethylation on the proximal promoter of the p65 gene mediated by the histone methyltransferase SET7/9. Transient HG, these authors propose, leaves an epigenetic dent on the *p65* gene such that even in the absence of the original stimulus endothelial dysfunction will sustain.

Circulating oxLDL presents a major risk to atherosclerosis in part by promoting the expression of adhesion molecules[24]. Fang et al. have recently reported a novel epigenetic mechanism underlying the induction of ICAM-1 in human endothelial cells[25]. oxLDL activated the transcriptional modulator MRTF-A, which in turn was recruited to the ICAM-1 promoter by p65 and synergistically stimulated ICAM-1 transcription with oxLDL. Depletion of MRTF-A erased H3/H4 acetylation and H3K4 dimethylation but restored H3K9 trimethylation on the ICAM-1 promoter. Since MRTF-A is known to engage the epigenetic machinery in regulating transcription within the vasculature[26-29], it is conceivable that MRTF-A may serve as the critical link of endothelial injury bringing histone modifying enzymes to the chromatin. Alternatively, Kim et al. propose that the regulatory subunit of the NAPDH oxidase complex p66shc mediates the induction of ICAM-1 by LDL[30]. LDL induced histone acetylation but inhibited DNA methylation of the p66shc promoter to up-regulate the transcription of p66shc. Of note, oxLDL has been reported to elicit epigenetic changes on a host of gene promoters in endothelial cells[31-33], although a genome-wide survey is lacking.

Chronic hypoxia has emerged as an independent

risk factor of atherosclerosis[34]. As a result of intermittent low oxygen supply, the transcriptome of endothelial cells undergoes marked changes that include an increase in the transcription rate of adhesion molecules[35]. Our laboratory has recently uncovered a potential role for Brg1 and Brm in hypoxia induced endothelium-leukocyte interaction. Expression of Brg1 and Brm in vitro was up-regulated in cultured endothelial cells exposed to 1% O_2 and in vivo in pulmonary arteries isolated from mice kept in a low-oxygen chamber for 4 weeks. Introduction of Brg1 and Brm into a Brg1/Brm-negative cell line (SW-13) greatly potentiated hypoxia-induced CAM transactivation whereas silencing of Brg1/Brm in endothelial cells crippled the effect of hypoxia. Brg1 and Brm formed a dynamic interaction with p65 on the CAM promoters where p65 recruits Brg1/Brm and Brg1/Brm reciprocally stabilizes p65. Brg1/Brm influenced CAM transactivation by altering histone H3/H4 acetylation and H3K4 methylation creating a friendly chromatin structure for the basic transcription machinery. More important, endothelial-specific targeting of Brg1 and Brm normalized CAM expression and attenuated hypoxia induced leukocyte adhesion in mice. Of intrigue, a similar strategy also alleviated the development of atherosclerotic lesions in *Apoe*$^{-/-}$ mice, indicating that Brg1 and Brm might be able to orchestrate endothelial injury in response to a range of different pro-inflammatory stimuli (unpublished observation).

EPIGENETIC REGULATION OF INDUCTION OF VASOACTIVE SUBSTANCES DURING ATHEROGENESIS

Endothelium derived NO plays a critical role in maintaining vascular integrity, the disruption of which contributes to atherogenesis[36]. Not surprisingly, eNOS expression can be down-regulated by multiple atheroprone factors[37]. oxLDL decreases acetylation of H3 and H4 and dimethylation of H3K4 while simultaneously increasing H3K9 trimethylation surrounding the eNOS promoter consistent with the repression of eNOS transcription in endothelial cells[25]. Again, MRTF-A appears to be the key coordinator of these epigenetic changes. In addition, there is a decrease of eNOS expression in mice deficient in LSD1, an H3K4/K9 demethylase, highlighting the role of histone methylation in fine-tuning eNOS transcription[38].

Fish et al. conducted a comprehensive survey of histone modifications on the eNOS promoter region in endothelial cells challenged with anoxia[39]. In the proximal region of the eNOS promoter (-166/-26), H3/H4 acetylation and H3K4 dimethylation declined as early as 1 hour following exposure to low oxygen. In contrast, these signature changes were not observed on the distal eNOS promoter (-891/-797 and -488/-398). A closer examination revealed that acetylation levels of specific lysine residues fluctuated with distinct patterns. For instance, 1 hour after hypoxia, only H3K14 and H4K5 acetylation plummeted significantly, which was joined by a decrease in H3K9/H4K8/H4K12 acetylation at 2 hours with H4K16 acetylation unaltered. Interestingly, histone H2A.Z was evicted from the proximal eNOS promoter during hypoxia thereby creating a closed chromatin conformation and rendering a repressed transcription state. Consistently, a recent study has correlated decreased eNOS expression with high levels of DNA methylation on the proximal eNOS promoter in patients with obstructive sleep apnea (OSA), a typical pathology of hypoxia[40]. Paradoxically, eNOS expression has been observed to increase, rather than decrease, in endothelial cells in response to hypoxia probably as means of compensation[41]. The up-regulation of eNOS transcription is accompanied by increased H3 and H4 acetylation across the eNOS promoter region (-4501/+23)[42]. Therefore, while it remains debatable how eNOS transcription responds to hypoxia, suffice it to say that a specific epigenetic code is intimately associated with the transcription status.

The vessel wall, particularly the endothelial layer, is subject to the pressure caused by various hemodynamic forces. Whereas laminar shear stress (LSS) is considered atheroprotective and stimulates eNOS transcription, turbulent blood flow creates shear stress that damages the vascular endothelium especially at the sites of arterial branches[43]. Among the detrimental effects exerted by pro-atherogenic shear stress are accelerated turnover of endothelial cells, increased adhesion of leukocytes, accumulation of reactive oxygen species, and decreased synthesis of NO stemming from eNOS repression[44-47]. Illi et al. have reported that LSS increased global H3 and H4 acetylation levels in endothelial cells through activating histone acetyltransferases (HATs)[48]. In addition, LSS augmented H3 and H4 acetylation on c-Jun and c-Fos promoters. Since c-Jun/c-Fos is considered essential for eNOS transactivation[49], increase AP-1 activity likely explains elevated eNOS transcription in response to LSS.

Impaired vessel relaxation during atherogenesis is also rooted in the enhanced expression of endothelin, a potent vasoconstrictor[50]. Accumulating evidence has provided a clear link between histone modification and ET-1 transactivation. In diabetic rats, increased ET-1 release was accompanied by an up-regulation of the histone acetyltransferase p300[51]. This observation

has been replicated in high glucose treated endothelial cells[52]. Silencing of p300 completely abolished ET-1 activation by high glucose thought it remained unclear whether p300 acted through histone or non-histone factors. Wort et al. have demonstrated that H4 acetylation was increased on the ET-1 promoter surrounding a conserved p65 binding element in endothelial cells treated with two pro-inflammatory stimuli, TNF-α and IFN-γ[53], suggesting a potential role for a HAT like p300. In rats with intrauterine growth retardation (IUGR) where hypoxia plays a determining role, H3K9/K18 and H4 acetylation increased on the ET-1 promoter[54]. Recently, our investigation has led to the identification of an MRTF-A-centered epigenetic complex on the ET-1 promoter in response to hypoxia[55]. Under hypoxic conditions, MRTF-A interacted with and was recruited by the sequence specific transcription factor SRF to the proximal ET-1 promoter (-81/+150). Upon the joining of Brg1/Brm, this epigenetic complex altered histone acetylation and H3K4 methylation to facilitate the binding of RNA polymerase II thereby activating ET-1 transcription.

FUTURE DIRECTIONS

The turn-of-the-century saw a boom in research on epigenetics with the initiation of several epigenomics projects[56]. Relying on high-fidelity chromatin immunoprecipitation (ChIP) coupled with high-throughput sequencing techniques, these projects aim to decode the epigenetic information bringing insights for the prevention and intervention of human diseases. Several exciting findings have provided clues for such basic biological events as adipogenesis[57], macrophage activation[58], and cell cycle progression[59]. So far, there has been a lack of effort in deciphering the epigenetic code on a genomewide scale in any of the major cardiovascular diseases including atherosclerosis. Since single-gene based epigenetic analysis tends to give very limited and often biased knowledge to the understanding of atherogenesis, long considered a multifactorial disease, basic scientists and clinicians like will benefit from an undertaking that unveils a comprehensive picture of epigenetic regulation of endothelial injury in atherosclerosis.

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