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Review

Epigenetic modulation of vascular diseases: Assessing the evidence and exploring the opportunities

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ABSTRACT

Vascular adaptations to either physiological or pathophysiological conditions commonly require gene expression modifications in the most represented cellular elements of the vessel wall, i.e. endothelial and smooth muscle cells. In addition to transcription factors, a number of mechanisms contribute to the regulation of gene expression in these cells including noncoding RNAs, histone and DNA modifications, collectively indicated as epigenetic modifications. Here, we summarize the state of art regarding the role of epigenetic changes in major vascular diseases, and discuss the potential diagnostic and therapeutic applications of epigenetic modulation in this context.

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death and disability in Western countries [1]. High morbidity and mortality of these prevalent diseases stem from the presence of a number of alterations affecting both heart and vessels homeostasis [1]. In particular, the vasculature is considered a very plastic system, and therefore the functional regulation of its constitutive elements, endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), is subjected to continuous adjustments in gene expression under either physiological or pathophysiological conditions [2].

Epigenetic mechanisms have emerged as one of the most important regulators of gene expression in eukaryotes [3]. Broadly defined as modifications of gene expression not involving changes of DNA sequence, epigenetic modifications can either increase or repress expression of specific genes in a time- and space-related manner. Epigenetic mechanisms include DNA methylation, histones post-translational modifications (HPTMs) and RNA-based mechanisms (Fig. 1). Presently,

Abbreviations: CVDs, cardiovascular diseases; ECs, endothelial cells; VSMCs, vascular smooth muscle cells; HPTMs, histones post-translational modifications; nt, nucleotides; 5-mC, 5-methylcytosine; 5-hmC, 5-hydroxymethylcytosine; CpG, sequence of cytosine followed by a guanine; DNMTs, DNA methyl-transferases; TET, ten-eleven translocation; k, lysine; me, methylation; HDACs, histone deacetylases; HDACi, HDACs inhibitors; UTR, untranslated region UTR; ncRNAs, non-coding RNAs; lncRNAs, long ncRNAs; miRs, microRNAs; mRNAs, messenger RNAs; eRNAs, enhancer RNAs; circRNAs, circular RNAs; eNOS, endothelial nitric oxide synthase; MMP, matrix metalloproteinase; TIMP1, tissue inhibitor of metalloproteinase 1; VEGF, vascular endothelial growth factor; SNPs, single nucleotide polymorphisms; RAAS, renin-angiotensin-aldosterone system; SHR, spontaneously hypertensive rats; Wistar Kyoto, WKY; oxLDL, oxidized low density lipoproteins; EndMT, endothelial-to-mesenchymal transition; PAD, peripheral artery disease; HAT, histone acetyl-transferase; ECFCs, endothelial colony-forming cells; AGEs, advanced glycation end products; AAA, abdominal aortic aneurisms; ECM, extracellular matrix; TGF-β, transforming growth factor-β; hypoxia-inducible factor-1α, (HIF-1α); IL-1β, interleukin-1β; PAH, pulmonary arterial hypertension; PASMCs, pulmonary artery smooth muscle cells; PAECs, pulmonary artery endothelial cells; T2D, type 2 diabetes; NAD^+, nicotinamide adenine dinucleotide; SIRTs, sirtuins; EPCs, endothelial progenitor cells

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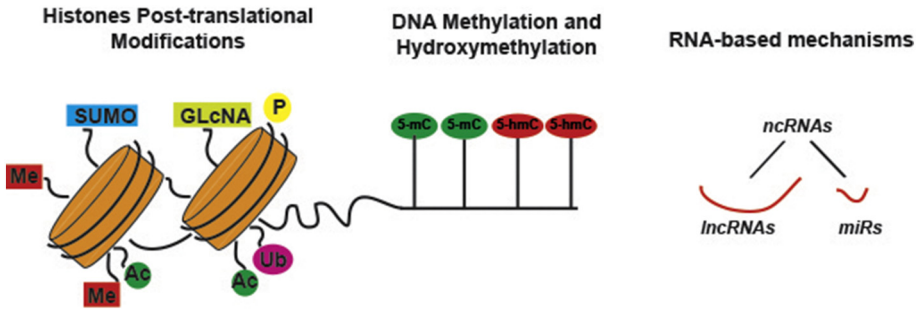

Fig. 1. Schematic representation of epigenetic mechanisms. Histone post-translational modifications (HPTMs), DNA methylation/hydroxymethylation and RNA-based mechanisms are major epigenetic changes identified regulating gene expression in eukaryotes. Abbreviations: SUMO, sumoylation; P, phosphorylation; Me, methylation; Ac, acetylation; Ub, ubiquitination; GLcNA, GLcNAcylation; 5-mC, 5-methylcytosine; 5-hmC, 5-hydroxymethylcytosine; ncRNAs, non-coding RNAs; lncRNAs, long ncRNAs; miRs, microRNAs.

the understanding of epigenetic mechanisms is far from being exhaustive [4] and is mostly limited to the nuclear/cytosolic environment whereas very little is known about mitochondrial epigenetics (reviewed in [5]). However, an increasing body of knowledge has recently emerged indicating epigenetic events important in the pathogenesis of CVDs [6]. Moving forward, others and we have suggested that manipulation of epigenetic processes might be useful for therapeutic purposes [4,7–9].

Here we provide a brief overview of epigenetic marks and examine the most recent evidence that establishes epigenetic events as fundamental mechanisms governing vascular function.

2. Epigenetics basics

Nuclear DNA is tightly packaged in DNA-protein complexes called chromatin [10]. The fundamental, repetitive unit of this structure is the nucleosome, consisting of 147 nucleotides (nt) of DNA wrapped around a core of eight histone proteins. Each octamer contains two copies of each histone H2A, H2B, H3 and H4. A fifth histone, H1, is associated with a short DNA sequence, which acts as a linker between sequential nucleosomes. The highly ordinated chromatin organization in nucleosomes is critical to enable the packaging of the entire DNA mass into the limited space of the cell nucleus. Despite this complex structure, chromatin is a highly dynamic environment in which structure modifications profoundly affect the accessibility of transcription factors to DNA, in order to modulate RNA synthesis. Both chromatin components, histones and DNA, can be subjected to epigenetic modifications that alter chromatin structure and accessibility [3].

2.1. DNA methylation and hydroxymethylation

Covalent addition of a methyl group to the 5′-position of the DNA base cytosine (5-methylcytosine, 5-mC) is a common epigenetic mark that has been associated with transcriptional repression [11]. DNA methylation occurs preferentially but not exclusively in the context of sequences of cytosine followed by a guanine (CpG), in what have been called CpG islands [12]. Three DNA methyl-transferases (DNMTs) catalyze methylation of cytosine across the genome: DNMT1, DNMT3a and DNMT3b [11]. While the former is involved in the maintenance of DNA methylation pattern during adult cell division, the latter two enzymes are mainly involved in the regulation of *de novo* DNA methylation during embryogenesis and organ development. DNA methylation can repress gene expression by either “physically” impeding the binding of transcription factors to the promoter region of the gene, recruiting proteins that specifically recognize the methyl-CpG groups and act directly as transcriptional repressors [13] or preventing the binding of activating factors [14]. Although DNA methylation has been traditionally considered a stable epigenetic mark, the recent discovery of 5-hydroxymethylcytosine (5-hmC) across the mammalian genome, has challenged the previous dogma. The evidence of active oxidation of 5-mC in 5-hmC by a family of enzymes called ten-eleven translocation (TET) has identified DNA hydroxymethylation as a novel epigenetic mark capable of regulating transcription by itself [15,16]. DNA hydroxymethylation seems to affect gene expression in both directions, acting as a repressor or activator of transcription depending by the context and gene [15]. The balance between DNA methylation and hydroxymethylation delineates the epigenetic landscape of DNA modifications.

2.2. Histone post-translational modifications (HPTMs)

Histone proteins can be subjected to a number of HPTMs, acting in concert to regulate transcription [17]. Acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, GLcNAcylation and biotinylation are only some of many already identified HPTMs [17], occurring primarily at the N-terminal tails of core histones. The complexity of this regulation is amplified by the contemporary presence of several different HPTMs, generating almost infinite combinations of signals. The identification of this “histone code” together with DNA epigenetic modifications defines the plethora of dynamic events that contribute to transcriptional control in mammals.

Among HPTMs, the best characterized are histone acetylation and methylation. Acetylation of the amino group of lysine (K) residues of histone H3 and H4 tails has been recognized to drive increased transcription, “opening” chromatin structure [17]. However, this more accessible configuration of chromatin can also recruit bromodomain-containing complexes (“readers” of acetylation marks), which can act indirectly as transcriptional repressors. In this context, enzymes mediating histone de-acetylation (histone deacetylases, HDACs) negatively affect transcription. The recently emerged availability of clinically approved HDACs inhibitors (HDACi) has further highlighted the paramount importance of these epigenetic modifications in human diseases [8].

The patterns of histone lysine methylation and the transcriptional effects of this epigenetic mark are more complex. Different methylation sites can result in repressive or permissive transcriptional chromatin configuration, and since the addition of methyl groups can be multiple on the same lysine a further level of complexity arises. Indeed, lysine 4, 9 and 27 of H3 can be mono-, di- or tri-methylated, and each of these modifications can differently affect chromatin structure. Similar to what has been observed for DNA methylation, also histone methylation was inaccurately considered a static epigenetic mark. It is now widely accepted that histone de-methylation is a dynamic process involving multiple histone de-methylases that can remove methyl groups from histone lysine in a gene-specific manner [18].

2.3. RNA-based epigenetics: non-coding RNAs

Another important category of epigenetic regulators of gene expression is represented by different classes of non-coding RNAs (ncRNAs), including short (< 200 nt) and long ncRNAs (lncRNAs, > 200 nt)[19]. Among the short ncRNAs, microRNAs (miRs) are the most largely studied. More than 2,500 miRs have been identified and although several families/clusters can be recognized, each miR can have multiple targets, governing the biology of a number of processes. Inhibition of gene expression by miRs occurs at post-