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Medicine in focus

The involvement of epigenetics in vascular disease development

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ABSTRACT

Cardiovascular diseases are a major cause of death and disability. Despite enormous progress in diagnosis, prevention, and treatment over the years, the incidence of this group of pathologies continues to increase worldwide. An important step in reversing this situation is filling in the gaps we have in our understanding of cardiovascular homeostasis and of the pathogenic processes leading to disease. On this point, the discovery of epigenetics – heritable chemical modifications of DNA bases and histone proteins, as well as non-coding RNA-based mechanisms regulating gene expression – has opened up new vistas. Here, we will review recent findings regarding the epigenetics of three main vascular diseases (atherosclerosis, restenosis, and aortic aneurysm), with a focus on DNA methylation and histone modification. The emerging fundamental nature of epigenetics for cardiovascular physiopathology and, importantly, the amenability to manipulation with pharmacological techniques are an indication that epigenetics-based prognostic and therapeutics procedures might be developed in the future.

1. Introduction

Cardiovascular diseases (CVDs) are the main cause of death and disability worldwide (Murray and Lopez, 1997). Despite enormous progresses in diagnosis, prevention, and treatment, which have considerably lowered incidence and improved overall survival, the number of people developing CVDs remains very high worldwide (Nabel and Braunwald, 2012). The strong penetrance of CVDs depends on a number of pathophysiological alterations influencing the heart and vasculature, such as those affecting the cardiovascular (CV) system's cells – which can fundamentally be narrowed down to cardiomyocytes, endothelial cells (ECs), and vascular smooth muscle cells (VSMCs) – and the reciprocal interactions between them (Climent et al., 2015; Tirziu et al., 2010). CV risk factors could translate into specific stresses acting on these cells and, more in depth, activate specific signaling pathways that eventually influence the cells' chromatin state, altering their gene expression profile. How chromatin structure affects gene expression is studied under the heading of epigenetics, a term coined to classify those heritable changes that, rather than depending on changes of the DNA sequence, are based on the chemical modification of DNA bases – in particular, cytosine – and histone proteins; more recently, processes regulated by non-coding RNAs have been added among those classified as epigenetic (Egger et al., 2004; Quintavalle et al., 2011).

As described in 1956 by the British developmental biologist Conrad Waddington, epigenetic-based mechanisms are essential modulators of cell fate determination (Waddington, 1956). In eukaryotes, specific cell development goes through coordinated events of activation and repression of the expression of specific genes in a precise time- and space-dependent manner in cells that share the same DNA sequence (Cantone and Fisher, 2013). Therefore, epigenetic inheritance is central for the stable propagation of gene activity states from one generation of cells to the next (Probst et al., 2009). Although the understanding of the epigenetic mechanisms associated with CVD development is still in its infancy, the definition of the epigenetic landscape in different pathological contexts is being facilitated by a dramatic improvement in DNA sequencing capacity paralleled by a big drop in costs. Thus, today it is possible to foresee how knowledge generated by studying the epigenetics of CVDs may be applied for therapeutic ends (van der Harst et al., 2017; Greco and Condorelli, 2015). In this review, we will focus on the chemical alteration of DNA and histones; for epigenetic mechanisms mediated by non-coding RNAs, we refer the reader elsewhere (Elia and Condorelli, 2015; Elia and Quintavalle, 2017; Thum and Condorelli, 2015).

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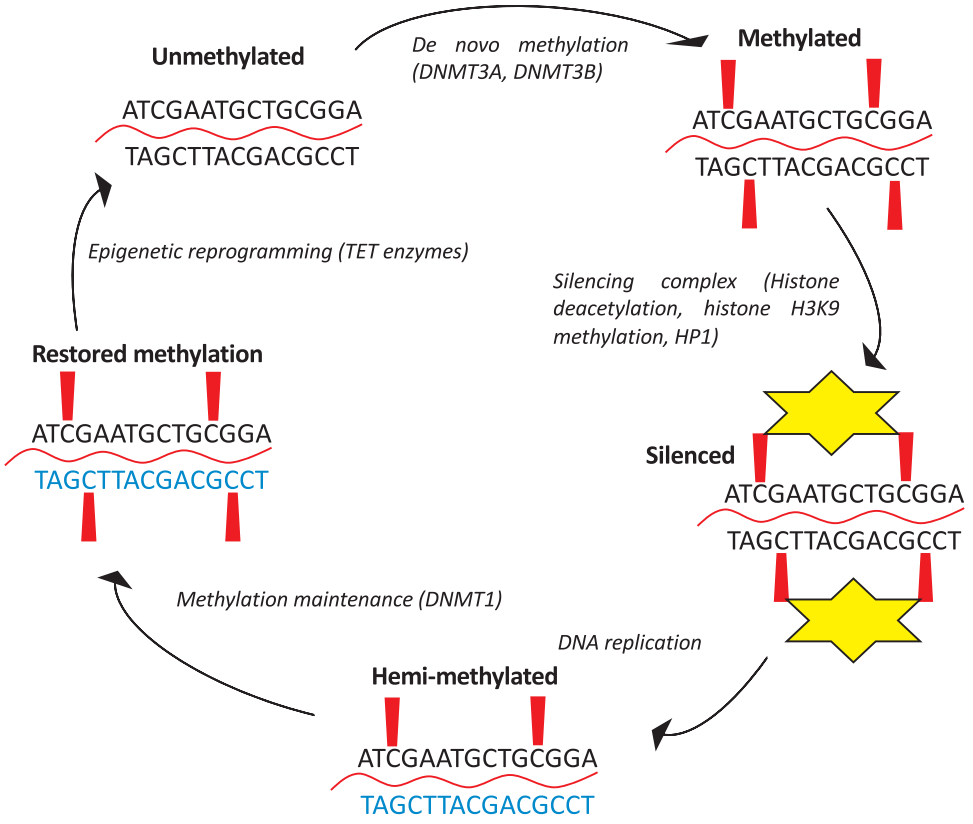

Fig. 1. DNA undergoes different chemical modifications during the differentiation process. De novo methylation (shown as red tags) is mediated primarily by DNA (cytosine-5-)-methyltransferase-3alpha (DNMT3A) and -3beta (DNMT3B). The DNA methylated at CpG islands is recognized by methyl-binding proteins (yellow stars), which trigger a silencing cascade whereby histone H3K9 is first deaceylated and then methylated, it being a binding substrate for heterochromatin protein 1 (HP1). Upon DNA replication, newly synthesized strands (shown in light blue) lack methylation marks, but DNMT1 rapidly deposits methyl groups on newly synthesized DNA, using the old DNA strand as a template for where to place the methyl groups. This results in the faithful conservation of methylation patterns needed to maintain gene silencing. Adult patterns of methylation are erased by epigenetic mechanism involving TET family proteins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2. Epigenetic alterations: DNA methylation and histone modifications

Nuclear DNA is tightly wrapped around a core of eight histone proteins (two copies each of H2A, H2B, H3, and H4), generating repetitive units named nucleosomes. These structures allow the DNA to be strongly compacted and contained in the limited space of the cell nucleus, through the formation of chromatin. Despite its complex three-dimensional structure, chromatin is a very dynamic entity: DNA and histones can be chemically modified so as to alter the accessibility of transcriptional factors to genes and, thus, modulate transcription (Goldberg et al., 2007). Only part of repertoire of post-translational modification of histone proteins has probably been unearthed to date; many of these involve histone protein H3, but modifications are also known to occur on H4, and less so on H2A and H2B.

DNA methylation occurs preferentially, but not exclusively, at specific dinucleotide sites along the genome where a cytosine base followed by a guanine – known as a CpG island – can be methylated to 5-methylcytosine (5-mC) (Bird, 1986) (Fig. 1). The enzymes catalyzing this modification are known as DNA methyltransferases (DNMTs): DNMT1 is involved fundamentally in maintenance of the methylation status of DNA during cell division, whereas DNMT3a and DNMT3b are essential for *de novo* methylation during developmental stages (Lister et al., 2009). The activity of DNMT1 has been demonstrated to be dependent on a co-factor that recognizes hemi-methylated DNA. Among these, ubiquitin-like, containing PHD and RING fingers domains, 1 (UHRF1) has been shown to be essential for maintenance of the methylation status of DNA by directing the recruitment of DNMT1 to replication forks (Bostick et al., 2007). In terms of biological significance, methylated CpG islands are always a marker of gene repression, since they act as docking sites for methyl-binding proteins. Indeed, they can generate steric impediment to the binding of a transcriptional factor to specific gene promoters, recruit transcriptional repressors, or prevent the binding of activator proteins (Prokhortchouk et al., 2001).

Besides methylation, other chemical modifications of cytosine have been identified, such as hydroxymethylation, formylation, and carboxylation, but their interrelation with methylation is not completely understood yet (Iurlaro et al., 2013). Among these further modifications, the discovery of 5-hydroxymethylation of cytosine (5-hmC) has challenged the traditional dogma that DNA methylation is a stable epigenetic mark. The active oxidation of 5-mC to 5-hmC is regulated by a family of proteins named ten-eleven translocation (TET) enzymes, and this modification seems to affect gene expression in both directions depending on the cellular context. Thus, hydroxymethylation is now considered a novel epigenetic mark of gene regulation that acts in concert with DNA methylation (Greco et al., 2016).

The epigenetic code on DNA is further complicated by the multiple chemical modifications taking place on histone proteins. These histone post-translational modifications (HPTMs) occur primarily at the amino acid residues on the core histones' N-terminal tails, which protrude from the chromatin fiber (Natsume-Kitatani et al., 2011). HPTMs allow binding to specific proteins, known as readers, that interfere with chromatin function to mainly modulate gene expression, but they can also affect apoptosis and DNA damage repair (Jenuwein and Allis, 2001). The complexity of such regulation is further amplified by the concomitant presence of differing HPTMs, creating numerous combinations of modifications (Fig. 2). Among the different HPTMs known, the most studied and defined are methylation and acetylation. Methyl is a very complicated epigenetic mark since it can signal for activation or repression of gene expression depending on the level of its deposition and on the specific histone involved. For instance, the lysine residues at positions 4, 9, and 27 on histone H3 can be methylated to different levels (mono-, bi-, and tri-methylation), resulting in different chromatin structures. Furthermore, histone methylation is a very dynamic process regulated by histone demethylases, which remove the methyl groups from lysine residues with a high gene specificity (Tsukada et al., 2006). These histone marks can revert very rapidly in response to diverse stimuli, and they may be involved in pathogenesis, as has been demonstrated for cardiac hypertrophy and failure (Papait et al., 2013, 2017).

In contrast, acetylation occurs mainly on lysine residues on histones

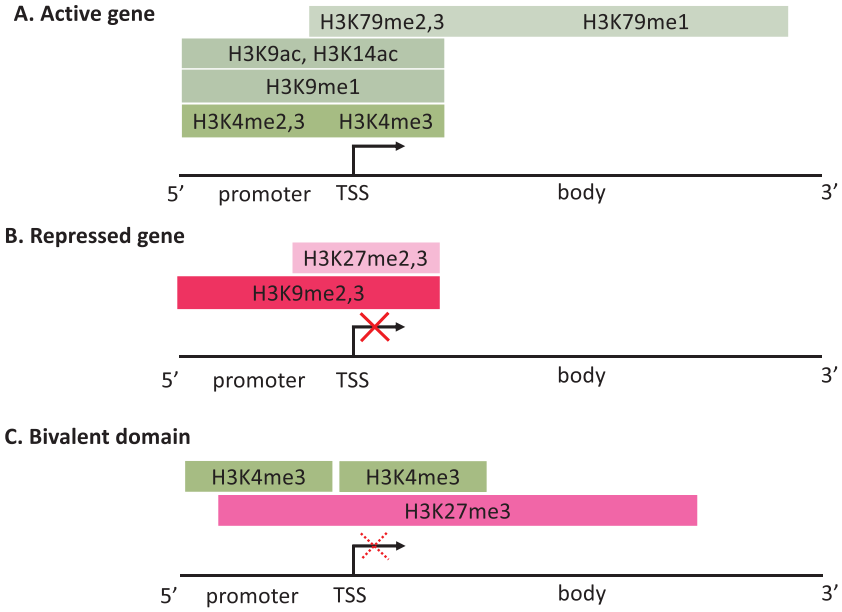

Fig. 2. Gene expression regulated by histone modification marks. Active marks are labelled in different shades of green, while shades of pink indicate repressive marks. (A) An example of a transcriptionally active gene characterized by specific patterns of histone acetylation and methylation marks at the promoter region, TSS (transcriptional start site), and gene body. (B) Histone H3 methylation is found at the promoter region and surrounding the TSS in transcriptionally repressed genes. (C) Bivalent chromatin domains consist of discrete pockets of H3K4me3 marks within large regions of H3K27me3 marks (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

H3 and H4; this mark is fundamentally associated with activation of transcription, enhancing accessibility to chromatin (Gillette and Hill, 2015). In this context, bromodomain-containing complexes are under considerable attention on account of their potential to bind acetyl-lysine and then to recruit histone deacetylases (HDACs), triggering gene repression (Haberland et al., 2009).

Dysregulation of the complex landscape of epigenetic modifications comprising DNA methylation patterns and HPTMs is being increasingly associated with pathological states, including CVDs (reviewed in (Greco and Condorelli, 2015; Muka et al., 2016; Zhong et al., 2016)).

3. Epigenetics and vascular diseases

The involvement of epigenetics in the development of all CVDs has been largely demonstrated. In particular, the environment strongly affects the epigenetic landscape and, therefore, gene expression in the vasculature, modulating EC and VSMC physiology. ECs are essential in creating a selective barrier between blood and the rest of the body's tissues. This fundamental role directly implicates endothelial dysfunction in many pathologies, including those of the CV system (Cai and Harrison, 2000). By contrast, VSMCs are responsible for the maintenance of vascular tone. Plasticity is a peculiarity of VSMCs: under physiological conditions, they can revert from a contractile phenotype to a migratory one for growth and wound healing (Owens, 1995). This feature, while physiological, could also contribute to the development of vascular pathologies, such as atherosclerosis, restenosis, and aortic aneurysm.

Atherosclerosis is a very complex pathology in which many factors and pathways are involved. From the clinical point of view, the disease is characterized by chronic inflammation affecting ECs and VSMCs, so contributing to the build-up of plaques that reduce the vascular lumen and motility (Glass and Witztum, 2001). Analysis of the methylation profile in human atherosclerotic plaques has demonstrated global DNA hyper-methylation, indicating an important positive correlation between DNA methylation and atherosclerosis development (Valencia-Morales Mdel et al., 2015; Zaina et al., 2014). For instance, the stimulation of ECs with oxidized low-density lipoproteins increases DNMT1 expression. This leads to methylation of the promoter region of the transcription factor Krüppel-like factor 2 (KLF2), inducing a pro-atherogenic EC phenotype (Kumar et al., 2013). In line with this finding, our group recently demonstrated that methylation modulated the atherogenic profile also of VSMCs: primary VSMCs treated with platelet-derived growth factor-BB strongly expressed UHRF1, which, in cooperation with DNMT1, positively modulated the methylation status of different genes associated with VSMC differentiation, such as smooth muscle actin 2, smooth muscle-myosin heavy chain 11, calponin, and transgelin (Elia et al., 2018).

In contrast, the role of histone methylation and acetylation in atherosclerosis development is still under debate, with observations of different behaviors occurring in cell dependent and independent manners. For instance, the level of the repressive mark H3K27me3 was found unchanged in ECs obtained from aortas of apolipoprotein E deficient (ApoE$^{-/-}$) mice fed a Western (*i.e.*, high fat) diet, whereas in the same model of atherosclerosis development there was a significant reduction of this histone modification in VSMCs (Alkemade et al., 2010). A more controversial observation was obtained for histone acetylation: EC-specific knock-down of HDAC3 in ApoE$^{-/-}$ mice was associated with an increased size of atherosclerotic plaques (Zampetaki et al., 2010). This contrasts with *in vitro* data demonstrating that overexpression of HDAC3 triggered repression in ECs of Klf4, promoting a pro-atherogenic phenotype (Lee et al., 2012).

Atherosclerosis mainly impacts patient lifespan because it is responsible for myocardial infarction. However, it is necessary to point out that this disease has other phenotypes that can be grouped under the name of peripheral artery disease (PAD) (Shammas, 2007). From the clinical point of view, PAD is much less responsive to classical therapies, such as endovascular approaches and anti-coagulant drugs, than is coronary occlusion. Typically, people diagnosed with PAD have important benefits from exercise training (Hamburg and Balady, 2011). The molecular reasons for this amelioration are unknown. However, since epigenetic changes, like DNA methylation and histone modifications, have been associated with exercise (Ling and Ronn, 2014), there is a strong argument for the involvement of epigenetics in PAD development (Kullo and Leeper, 2015). Despite this, few studies have investigated the influence of epigenetics in PAD pathogenesis to date (reviewed in (Golledge et al., 2016)). For instance, in a murine model of femoral artery ligation, therapy with a histone deacetylase inhibitor (HDACi) delayed maturation of newly formed muscle fibers, with consequential increased compensatory fibrosis and muscle atrophy (Spallotta et al., 2013). This can be explained by the fact that the histone acetyl-transferase p300 triggers migration and, therefore, the vascular repair capacities of human endothelial colony-forming cells (Palii et al., 2014). However, the negative impact of HDACi in this *in vivo* PAD model was not in line with the beneficial effects that these

compounds have in coronary artery disease (Gillette and Hill, 2015). Therefore, further studies are warranted.

Another important vascular pathology is restenosis, an iatrogenic disease caused mainly by EC damage in a coronary artery following stent placement. The absence of endothelium increases adherence of circulating inflammatory cells to the vessel, depriving the damaged organ of an anti-proliferative brake function on VSMCs (Rajendran et al., 2013). Epigenetics plays an important role in restenosis, acting directly on VSMC gene expression. In this context, VSMC DNA methylation status plays a pivot role (Zhuang et al., 2017). Indeed, it was shown that TET2, which promotes the formation of 5-hmC, enhanced the activation of contractile genes in VSMCs; moreover, local knockdown of TET2 exacerbated the response to vascular injury *in vivo* (Liu et al., 2013). An inverse approach confirmed the direct association between restenosis and DNA methylation: as reported above, UHRF1 is involved in the regulation of the contractile status of VSMCs, and indeed its local modulation or genetic elimination strongly inhibited restenosis in mouse carotid artery (Elia et al., 2018). Similarly, modulation of histone acetylation might impact restenosis development since HDACi reduced VSMC proliferation *in vitro* and blunted neointimal formation *in vivo* (Findeisen et al., 2011).

Aortic aneurysm is another CV pathology in which epigenetics could play a role. This disease can be driven genetically in a monogenic context, like in Marfan syndrome for instance (Judge and Dietz, 2005), or in a complex disease context, such as in those aortic aneurysms having a much broader and more intricate etiopathogenesis (Kuivaniemi et al., 2015). In the latter, a critical role is played by the balance between extracellular matrix (ECM) deposition and degradation (Didangelos et al., 2011). A shift toward ECM degradation is associated with vascular fragility: indeed, expression of matrix metalloproteinases (MMPs) 2 and 9 has been reported increased in aortic aneurysms (Rabkin, 2017). More recently, it was demonstrated that these MMPs are epigenetically controlled by histone acetylation in human aneurysms (Galan et al., 2016; Zhong and Kowluru, 2013).

In line with findings on the role of ECM metabolism, the development of aortic aneurysm depends also on VSMC differentiation status. Indeed, differentiated VSMCs contract, maintaining the vascular tone and producing ECM. As discussed for restenosis, DNA methylation strongly regulates the contractility of VSMCs (Zhuang et al., 2017); our group recently demonstrated that modulating the methylation level in VSMCs through deletion of UHRF1 strongly improved vascular response to the induction of aneurysms in ApoE$^{-/-}$ mice with the infusion of angiotensin II (Elia et al., 2018).

4. Conclusions

The above-mentioned studies strongly concur in suggesting that epigenetics is fundamentally involved in the pathogenesis of CVDs. A better understanding of the complex mechanisms linking epigenetics and CVDs will probably have significant consequences on many aspects of CV medicine, from prevention to prognosis and therapy.

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Disclosure

None.

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