Impact of epigenetics in the management of cardiovascular disease: a review

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Abstract. – Cardiovascular disease (CVD) is the leading cause of death, irrespective of socioeconomic status, ethnic background and sex. Despite the considerable progress in the treatment, the complex pathophysiology underlying CVD is still not clear. In past few years, genetic approaches including epigenetics and personalized medicine initiated a new way of treating CVD. Epigenetics refers to the non-DNA sequence related heritable changes in gene expression and its role in understanding and treating coronary artery disease, heart failure, and cardiac hypertrophy is currently recognized as an important player. Histone acetylation, deacetylation, DNA methylation and histone methylation are different mechanisms of epigenetic modifications. Cardiac Hypertrophy is linked with histone acetylation and the activity of histone acetyltransferases (HATs) has a positive role in cardiac hypertrophy. Altered DNA methylation, miRNA activity have been shown to be associated with atherosclerosis. It is documented that re-expression of certain fetal genes in the adult heart contributes to the development of heart failure syndrome, which is often associated with pathological cardiac remodeling comprising of changes in heart mass, size and shape. Thus, it appears that approaches that counteract epigenetic changes occurring in CVD can prove to have significant therapeutic impact. However, there are no major clinical practice or therapeutics reports of epigenetics contribution in CVD, even though deacetylase inhibitors like trichostatin A were shown to have some positive effects.

In this review we will present an overview of various epigenetic mechanisms such as DNA methylation, histone modifications, and microRNA-dependent mechanisms in CVD and the novel epigenetics-based therapeutic approaches.

Key Words: Epigenetics, Cardiovascular disease, Histone, microRNA.

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide despite significant disparities related to socioeconomic strata and gender. Several pathological conditions that afflict heart including myocardial infarction, cardiac hypertrophy, myocardial ischemia, coronary artery disease and hypertension, lead to heart failure (HF). However, the complex pathophysiology that leads to CVD is far from clear and alternate mechanisms are constantly looked for in order to provide better and collective explanations. Several molecular and cellular mechanisms underlie the development of HF that includes reprogramming of expression of certain critical cardiac genes including downregulation of the alpha-myosin heavy chain (2-MHC) gene and sarcoplasmic reticulum Ca+ATPase genes and reactivation of specific fetal cardiac genes such as atrial natriuretic factor and brain natriuretic peptide.

Over the past few years, advancements in the areas of epigenetics and epigenomics have offered a new and different look at human diseases and thus initiated a new era in genetic medicine by exploring the role of genetic heritability and environmental interaction in disease pathology. Heritability of CVD can vary depending on sex and environmental and lifestyle conditions. Genetic contribution to CVD can vary widely, as much as 40% to 80% and environment-gene interactions (potentially mediated by epigenetics) can affect the prevalence of CVD. Epigenetics refers to mechanisms related to the regulation of gene expression, which are chromatin-based but not involving any DNA sequence changes. There is significant experimental evidence suggesting that epigenetic modifications serve as a cellular...
memory of exposure in early life to unsuitable environments. It has been noticed that even among MZ twins, who are epigenetically indistinguishable in early life, environment and lifestyles can greatly influence epigenetic patterns, with age. These differences affect single-copy genes and repeat sequences. Anthropomorphic features, and vascular branching patterns are few examples of how disease susceptibility might vary between MZ twins. Epigenetic modifications are reversible, vary between cell types, and respond to endogenous and exogenous stimuli, as well as environmental signals (Figure 1). Moreover, these modifications can elicit long-term changes in gene transcription that potentially lead to disease susceptibility in later life.\(^9\)

**Epigenetic Mechanisms and their Implications in CVD**

Epigenetics comprises of three distinct processes that are interrelated. These are (1) DNA base modifications, (2) the histone modifications and (3) RNA-based mechanisms (Figure 1).\(^{11}\)

### DNA Base Modifications

Cytosine methylation is the major pathway by which DNA is modified that is relevant for epigenetic changes. DNA is made up of complementary pairs of the 4 nucleotides (guanine, cytosine, thymine, and adenine) running in various sequences along two opposing DNA strands. Cytosine methylation is conducted by DNA methyltransferases (DNMTs), which transfer a methyl group to the position-5 of the nitrogen base cytosine ring. Approximately 0.6-1.5% of the human genome is contributed by 5-methylcytosine (5 mC), even though its levels differ between different tissue types.\(^{12}\) Altered regulation of cytosine methylation has been linked to tumour cell development and CVD.\(^{13}\) Methylation of cytosines at promoter sites is associated with long-term transcriptional repression of the corresponding gene by directly blocking the binding of specific transcription factors to their cis-DNA elements. Even though this mechanism is relevant for transcriptional regulators like Myc, AP2, hypoxia-inducible factor-1, and

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**Figure 1.** The interrelation of three distinct processes: (1) DNA base modifications, (2) the histone modifications and (3) RNA-based mechanisms.
the insulator protein CTCF, DNA methylation does not universally affect the binding of all trans-factors (e.g., SP1)\textsuperscript{14}.

Altered methylation patterns and extent of regulatory regions of genes is known to alter their expression and correlate with failing hearts. Three angiogenesis-related genes were found to be differentially methylated, irrespective of the etiology of HF. These are: hyper-methylation of the 5\textsuperscript{\textprime}-regulatory region of platelet endothelial cell adhesion molecule-1 and hypo-methylation of the angiomotin like protein-2 is noted in failing hearts and this is associated with reduced expression of these genes. On the other hand, hyper-methylation within the Rho-GTPase activating protein-24 gene in failing hearts is correlated with elevated expression of this gene\textsuperscript{15}. Moreover, a follow up study\textsuperscript{16} generated a genome-wide DNA methylation map of human hearts and showed significantly lowered global promoter methylation of genes with increased expression in failing hearts. Moreover, DNA methylation can lead to hypermutability of distinct cardiac genes, like the cardiac isoform of the myosin binding protein C gene (Mybpc3), which has higher level of exonic methylation of CpG sites as compared to the skeletal muscle isoform\textsuperscript{17}. Clinical studies involving a follow up of elderly subjects showed that hypomethylation of long interspersed nucleotide elements was associated with higher incidence of ischemic heart disease events\textsuperscript{18}.

Associations have also been made between altered DNA methylation and atherosclerosis and vascular inflammation. Deficiency of folic acid, which is important factor in ‘One-carbon’ metabolism and as the carrier of methyl groups for methylation reactions, has been epigenetically linked to endothelial dysfunction and different aspects of CVD, including coronary heart disease, anemia, atherosclerosis, and stroke\textsuperscript{19}. Similarly, homocysteinauria has been associated with the suppression of angiogenesis, VSMC proliferation, dyslipidemia, vascular oxidative stress, and impairment of endothelial regeneration and function\textsuperscript{20}. It has been suggested that dysregulation of DNA methylation during different stages of embryonic growth might lead to congenital heart disease, improperly functioning vascular tissue, along with increased future risk of CVD as a result of the improper imprinting of genes, or inappropriate silencing of pluripotent or tissue-specific gene expression patterns\textsuperscript{21}. Even at very early stages of atherosclerosis, much before the anatomical manifestation of the disease are noticed, hypomethylation could be detected\textsuperscript{22}. Estrogen receptor (ESR1) and estrogen receptor (ESR2), which are atheroprotective in vascular smooth muscle cells, were found to be hypermethylated in human atherosclerosis\textsuperscript{23}.

**Cytosine Hydroxymethylation**

Besides methylation, many genes, intergenic regions, and repetitive elements are also hydroxymethylated in the heart, leading to altered expression of these genes\textsuperscript{24}. For example, lowered expression in association with hypermethylation and a dysregulation of hydroxymethylation patterns of the gene coding for epidermal growth factor receptor (EGFR), has been found to cause abnormal valve differentiation in the heart, leading to aortic stenosis and left ventricular hypertrophy\textsuperscript{25}.

**Histone Modifications**

Histones are the basic proteins around which DNA is wound in chromatin and these proteins make up nucleosomes. There are 8 core histone proteins in nucleosomes: two dimers of H2A/H2B and two dimers of H3/H4. Each core histone has an amino-acid tail that can be modified. Posttranslational modifications to the N-terminal of these tails can modulate histone-DNA interactions and are important players in epigenetic regulation of gene expression by influencing chromatin structure and controlling accessibility of transcriptional regulators to cis-DNA binding elements\textsuperscript{14}. Common modifications include lysine acetylation and methylation, arginine methylation, and serine phosphorylation\textsuperscript{26}. Histone acetylation is catalyzed by proteins known as histone acetyltransferases (HATs) and histone deacetylation is carried out by HDACs. Similarly, histone methylation levels are regulated by histone methyltransferases and histone demethylases.

Some of the transcription factors, and their co-activators and repressors possess catalytic activity of either histone acetylation or deacetylation. Histone acetylation at the lysine residues of the histone tails results in chromatin de-condensation and also provides a binding site for bromodomain proteins and transcriptional activators, leading to transcriptional activation\textsuperscript{27}. On the other hand, histone deacetylation causes reverse changes, inducing chromatin condensation and transcriptional repression\textsuperscript{28} (Figure 1). In experimental animal models, cardiac hypertrophy (CH)
has been linked with histone acetylation implicating both histone acetyltransferases (HATs) and histone deacetylases (HDACs). It has been shown that hypertrophic growth of cardiomyocytes could be induced by the overexpression of transcriptional co-activators CREB binding protein (CRB) or p300, individually, in a manner that is dependant on their HAT activity. Thus, inhibition of these co-activators could repress phenylephrine-induced hypertrophic cell growth. Overexpression of CBP or p300 with catalytic activity, in cardiomyocytes, leads to hypertrophy, whereas the corresponding mutant forms lacking HAT activity did not produce such effects.

The activity of HDACs has also been implicated in both pro- and anti-hypertrophic pathways, resulting in conflicting data and further work is needed to completely understand the role of these enzymes in CH. The two classes of HDACs were found to show opposing effects on cardiac hypertrophy, with class I HDACs being pro-hypertrophic and class IIa HDACs being antihypertrophic. Elevated expression of HDAC2 in cardiomyocytes mimics hypertrophic growth in an Akt-dependent manner. Class I HDAC2 overexpressing transgenic mice were shown to develop cardiac hypertrophy whereas HDAC2-null mice were protected from pressure overload or isoproterenol induced cardiac hypertrophy.

Histone methylation plays an important role in the pathogenesis of CVD. In a genome-wide study of histone methylation in heart, altered trimethyllation of histones H3H4 and H3K9 has been reported that in HF. Cardiac specific knockout of Dot1L in mice, which catalyzes H3K79 methylation in mammals, leads to a phenotype similar to dilated cardiomyopathy. Dilated cardiomyopathy is a leading cause for HF causing about one third of HF cases. A histone trimethyl demethylase, JMJD2A, is upregulated in cardiomyopathy patients and cardiac-specific overexpression of JMJD2A in mice causes extensive cardiac hypertrophy as compared to control mice, following transverse aortic constriction. However, jmjd2a-KO mice are protected from cardiac stress induced by transverse aortic constriction.

One of the important endothelial gene that is relevant for angiogenesis and CVD that is regulated by the histone modifications is NOS3, that codes for endothelial nitric oxide synthase (eNOS), which forms NO from L-arginine in blood vessels. Under normal physiological conditions, the NOS3 gene is transcriptionally active in endothelial cells, but repressed transcriptionally in VSMCs. Under hypoxic conditions, there is a substantial decrease in NOS3 transcription in endothelial cells, probably due to eviction of histones, including those associated with transcriptional activation (acetyl H3K9, methyl H3K4, acetyl H4K12), at the NOS3 proximal promoter site. Inhibition of histone deacetylation by trichostatin A (TSA) in vivo in mouse models, was shown to prevent myocardial remodelling and to reduce myocardial and serum tumour necrosis factor alpha levels and to greatly improved functional myocardial recovery after myocardial infarction. HDAC inhibition also enhanced the formation of myocytes and microvessels in the heart, in combination with increased cytokinesis. These findings suggested that HDAC inhibition can protect from heart failure by minimizing a loss in myocardial performance after MI by promoting angiogenesis.

### RNA-based Mechanisms

Among the three general modes of epigenetic regulation, RNA-based mechanisms are relatively the most recently described and much works needs to be done to gain clear understanding of these mechanisms. Current research focuses on noncoding RNAs and small RNAs (i.e., RNAs that do not code for any particular protein) that function in the nucleus to regulate gene transcription. In particular, long noncoding RNAs (lncRNAs), that are longer than 200 nucleotides, play an important role in epigenetic regulation. Inside the nucleus RNA-based epigenetic regulation is mostly achieved by lncRNAs, whereas small noncoding RNAs control posttranscriptional processes (translation and RNA degradation) in the cytoplasm, even though some small noncoding RNAs were found to play a role in chromatin-based silencing.

Micro-RNAs (miRNA) have emerged recently as important players of epigenetic regulators of gene expression (Figure 1). MiRNA biogenesis involves formation of several thousands of primary miRNA (pri-miRNA) through the RNA polymerase II-mediated transcription of miRNA encoding regions. The hairpin-shaped transcript is processed by Drosha/DGCR8 complex to produce precursor miRNA (pre-miRNA), with a stem-loop structure of about 70-100 nucleotides in length. Then pre-miRNA is transported by exportin-5 from nucleus to the cytoplasm, where the enzyme Dicer cleaves the pre-miRNA into a short double-stranded RNA frag-
ment of 20-25 nucleotides long called miRNA:miRNA duplex. The miRNA is incorporated into the RNA-induced silencing complex (RISC), in which the miRNA is unwound and split into two single strands, followed by the retention of the mature miRNA single strand, determining the formation of miRISC. The miRISC complex recognizes and regulates specific target mRNA. MiRNAs are present in multiple copies and lead to translational suppression of target mRNA, by transcript degradation and negative regulation of post-transcriptional events. Key miRNAs regulate the expression of several genes at the same time, and many miRNAs cooperatively regulate their target genes."42.

More than a thousand different miRNAs have been described in humans. Complete knowledge of miRNAs biology in the cardiovascular system under physiological and pathological conditions appears to be important for CVD prevention, diagnosis and therapy."43. It has been shown that overexpression of miR-23a, miR-23b, miR-24, miR-195 or miR-214 in neonatal cardiomyocytes induced CH, whereas overexpression of miR-133 inhibited the phenotype."44,45. MiRNAs play important role in programming and modulating gene expression of different cell types that participate in atherosclerosis. They mediate inflammation, cholesterol influx, cellular differentiation and lipid uptake. Distinct expression signatures of miRNAs in healthy and failing hearts has been noted and the differential miRNA expression among failing hearts is dependent on the underlying HF etiology."46. Thus, nearly 14 aortic stenosis-specific miRNAs were found while a set of another eight miRNAs, mainly expressed in a cardiomyopathic form of HF, were noticed."46.

The expression levels of miRNAs (Table I) were found to vary with the progression of disease. Global miRNA profiles in a double transgenic mouse model, harboring mutations in both the myosin heavy chain gene and the cardiac troponin I gene, resulting in severe HCM and premature mortality by age of 21 days, revealed stable downregulation of miR-1 and miR-133 at age of 10 and 16 days, suggesting a functional role for these miRNAs throughout the progress-

Table I. Altered expression of various miRNAs in cardiac abnormalities.

<table>
<thead>
<tr>
<th>Cardiac Condition</th>
<th>mi-RNA</th>
<th>Regulation</th>
<th>Species/ Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Myocardial Infarction</td>
<td>miR-21</td>
<td>Reduced levels in infarcted areas</td>
<td>Rat myocytes</td>
</tr>
<tr>
<td></td>
<td>miR-208</td>
<td>Elevated</td>
<td>Rat, human plasma; human heart tissue</td>
</tr>
<tr>
<td></td>
<td>miR-1</td>
<td>Elevated</td>
<td>Rat myocytes &amp; human serum</td>
</tr>
<tr>
<td></td>
<td>miR-208b; miR-499</td>
<td>Elevated</td>
<td>Human plasma</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>miR-133</td>
<td>Upregulated</td>
<td>Human plasma</td>
</tr>
<tr>
<td></td>
<td>miR-499</td>
<td>Upregulated</td>
<td>Human plasma</td>
</tr>
<tr>
<td></td>
<td>miR-340 &amp; 624</td>
<td>Upregulated</td>
<td>Human platelets</td>
</tr>
<tr>
<td>Heart Failure</td>
<td>miR-214</td>
<td>Elevated</td>
<td>Human left ventricle</td>
</tr>
<tr>
<td></td>
<td>miR-499</td>
<td>Elevated</td>
<td>Human Plasma</td>
</tr>
<tr>
<td></td>
<td>miR-24</td>
<td>Upregulated</td>
<td>Human left ventricle</td>
</tr>
<tr>
<td></td>
<td>miR-497</td>
<td>Down-regulated</td>
<td>Human PBMCs</td>
</tr>
</tbody>
</table>
sion to HF. On the other hand, miR-31 levels increased at the end-stage of HF, suggesting a specific function for this miRNA during the final phase of the disease. MiRNA expression profiles (Table I) were shown to differ between healthy and failing hearts, and that failing adult hearts and fetal hearts display similar miRNA profiles supporting the paradigm of reactivation of a fetal gene program at the beginning or during the development of HF.

**ATP-Dependent Chromatin-Remodeling**

ATP-dependent chromatin-remodeling complexes regulate gene expression by regulating nucleosome distribution on DNA, in an ATP-dependent manner. Nucleosomal association with DNA in turn controls the binding of transcription factors, which can bind to nucleosome core free DNA regions. Chromatin-remodeling complexes are divided into four families, viz., SWI/SNF, ISWI, CHD, and INO80, based on their ATPase subunits. Brahma-associated factor (BAF) complex, the mammalian ortholog of SWI/SNF complex, identified initially in Saccharomyces cerevisiae, consists of 14 BAF subunits encoded by 25 genes. These subunits can be assembled to form a hundred different BAF complexes, which have either brahma (Brm) or brahma-related gene 1 (Brg1) as the ATPase subunit and BAF complex is implicated in multiple processes, including heart and muscle development.

Brg1 deletion from endocardial cells during early stages of development leads to morphological defects of the heart due to deranged myocardial trabeculation. Also, it has been shown that BAF180 subunit is necessary for normal coronary and heart chamber development.

Brg1 is expressed in embryonic heart but not in adult cardiomyocytes. Brg1 is instrumental in the switch from the expression of fetal myosin heavy chain (i.e., b-MHC) to that of adult MHC (a-MHC) isoform during cardiac hypertrophy. However, Brg1 expression is induced by pro-hypertrophy stimuli; Brg1 then interacts with its partners HDAC and PARP, forming complexes that cause the pathological shift from adult to fetal MHC isoforms. Recent studies showed that BRG1-KO mice are more resistant to a pro-hypertrophic stimuli, such as transverse aortic constriction. Also, expression level of Brg1 was found to be correlated with the severity of cardiomyopathy in the hearts of hypertrophic cardiomyopathy patients.

**Conclusions**

Heart failure, a leading cause of morbidity and mortality, is the final outcome of different pathological conditions of heart due to complex genetic predisposition and environmental factors, and is accompanied by pathological cardiac remodeling, i.e., changes in heart mass, size, and shape. Many recent studies support an important role of epigenetics in the pathogenesis of heart failure and cardiac hypertrophy. Despite significant research, still clear answers are not available for: (1) the genomic regions that are regulated by epigenetic mechanisms; (2) the interactions and biological implications of the various epigenetic mechanisms including DNA methylation, histone modifications, chromatin remodeling and miRNAs; (4) the impact of epigenetic modifications on the function of non-cardiomyocyte cells (e.g., cardiac fibroblasts and endothelial cells) of heart tissue during heart failure; and (5) what is the interaction between environment (e.g., diet, smoking, stress) and epigenetic changes in the etiology of heart failure. One mechanism of gene expression regulation that has gained importance is epigenetics. Counteracting the gene expression changes occurring in heart failure could be a therapeutic approach. Even though epigenetic pathways have been therapeutically targeted for cancer treatment, such efforts are still in infancy for heart failure.

**Conflict of Interest**

The Authors declare that there are no conflicts of interest.

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